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24 OCT 2002
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# Request for grant of a patent

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1. Your reference — P015257GB DAA

2. Patent application number *(The Patent Office will fill in this part)* — 24 OCT 2002 — 0224830.0

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

   Sterix Limited
   The Magdalen Centre
   Robert Robinson Avenue
   Oxford Science Park
   Oxford OX4 4GA

   07658134001
   Patents ADP number *(if you know it)*

   If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — COMPOUND

5. Name of your agent *(if you have one)* — D Young & Co

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   21 New Fetter Lane
   London
   EC4A 1DA

   Patents ADP number *(if you know it)* — 59006 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
|---|---|---|
| | | |

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing *(day / month / year)* |
|---|---|
| | |

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   *a) any applicant named in part 3 is not an inventor, or*
   *b) there is an inventor who is not named as an applicant, or*
   *c) any named applicant is a corporate body.*
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| Priority documents | 0 |
| Translations of priority documents | 0 |
| Statement of inventorship and right to grant of a patent *(Patents Form 7/77)* | 0 |
| Request for preliminary examination and search *(Patents Form 9/77)* | 0 |
| Request for substantive examination *(Patents Form 10/77)* | 0 |
| Any other documents *(please specify)* | 0 |

11. I/We request the grant of a patent on the basis of this application.

Signature D Young & Co Date 24 October 2002

D Young & Co (Agents for the Applicants)

12. Name and daytime telephone number of person to contact in the United Kingdom: David Alcock 023 8071 9500

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# COMPOUND

## FIELD OF INVENTION

The present invention relates to a compound. In particular the present invention provides compounds capable of inhibiting 11β-hydroxysteroid dehydrogenase (11β-HSD).

## Introduction

**The role of glucocorticoids**

Glucocorticoids are synthesised in the adrenal cortex from cholesterol. The principle glucocorticoid in the human body is cortisol, this hormone is synthesised and secreted in response to the adrenocortictrophic hormone (ACTH) from the pituitary gland in a circadian, episodic manner, but the secretion of this hormone can also be stimulated by stress, exercise and infection. Cortisol circulates mainly bound to transcortin (cortisol binding protein) or albumin and only a small fraction is free (5-10%) for biological processes [1].

Cortisol has a wide range of physiological effects, including regulation of carbohydrate, protein and lipid metabolism, regulation of normal growth and development, influence on cognitive function, resistance to stress and mineralocorticoid activity. Cortisol works in the opposite direction compared to insulin meaning a stimulation of hepatic gluconeogenesis, inhibition of peripheral glucose uptake and increased blood glucose concentration. Glucocorticoids are also essential in the regulation of the immune response. When circulating at higher concentrations glucocorticoids are immunosuppressive and are used pharmacologically as anti-inflammatory agents.

Glucocorticoids like other steroid hormones are lipophilic and penetrate the cell membrane freely. Cortisol binds, primarily, to the intracellular glucocorticoid receptor (GR) that then acts as a transcription factor to induce the expression of glucocorticoid responsive genes, and as a result of that protein synthesis.

**The role of the 11β-HSD enzyme**

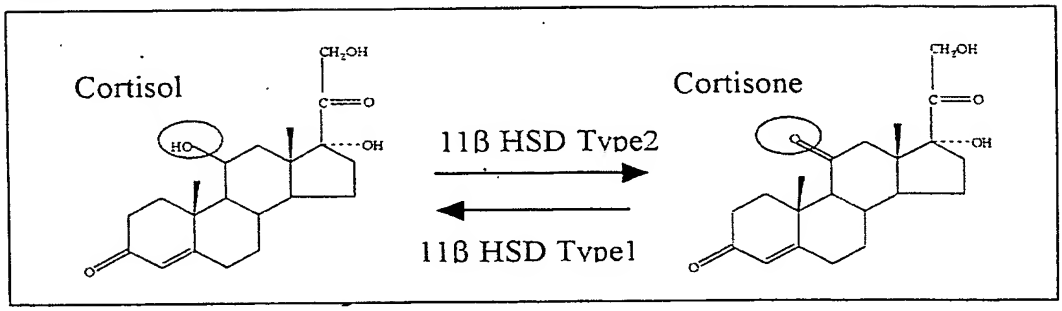
The conversion of cortisol (F) to its inactive metabolite cortisone (E) by 11β-HSD was first described in the 1950's, however it was not until later that the biological importance for this conversion was suggested [2]. In 1983 Krozowski et al. showed that the mineralocorticoid receptor (MR) has equal binding affinities for glucocorticoids and mineralocorticoids [3]. Because the circulating concentration of cortisol is a 100 times higher than that of aldosterone and during times of stress or high activity even more, it was not clear how the MR remained mineralocorticoid specific and was not constantly occupied by glucocorticoids. Earlier Ulick et al. had described the hypertensive condition known as, "apparent mineralocorticoid excess" (AME), and observed that whilst secretion of aldosterone from the adrenals was in fact low the peripheral metabolism of cortisol was disrupted. These discoveries lead to the suggestion of a protective role for the enzymes. By converting cortisol to cortisone in mineralocorticoid dependent tissues 11β-HSD enzymes protects the MR from occupation by glucocorticoids and allows it to be mineralcorticoid specific. Aldosterone itself is protected from the enzyme by the presence of an aldehyde group at the C-18 position.

Congenital defects in the 11β-HSD enzyme results in over occupation of the MR by cortisol and hypertensive and hypokalemic symptoms seen in AME.

Localisation of the 11β-HSD showed that the enzyme and its activity is highly present in the MR dependent tissues, kidney and parotid. However in tissues where the MR is not mineralocorticoid specific and is normally occupied by glucocorticoids, 11 β-HSD is not present in these tissues, for example in the heart and hippocampus [5]. This research also showed that inhibition of 11 β-HSD caused a loss of the aldosterone specificity of the MR in these mineralocorticoid dependent tissues.

It has been shown that two iso-enzymes of 11 β-HSD exist. Both are members of the *short chain alcohol dehydrogenase* (SCAD) superfamily which have been widely conserved throughout evolution. 11 β-HSD type 2 acts as a dehydrogenase to convert the secondary alcohol group at the C-11 position of cortisol to a secondary ketone, so producing the less active metabolite cortisone. 11 β-HSD type 1 is thought to act mainly in vivo as a reductase, that is in the opposite direction to type 2 [6] [see below]. 11 β-

HSD type 1 and type 2 have only a 30% amino acid homology.




**11 β-HSD enzyme activity**

The intracellular activity of cortisol is dependent on the concentration of glucocorticoids and can be modified and independently controlled without involving the overall secretion and synthesis of the hormone.

**The role of 11 β-HSD Type 1**

The direction of 11 β-HSD type 1 reaction in vivo is generally accepted to be opposite to the dehydrogenation of type 2. In vivo homozygous mice with a disrupted type 1 gene are unable to convert cortisone to cortisol, giving further evidence for the reductive activity of the enzyme [7]. 11 β-HSD type 1 is expressed in many key glucocorticoid regulated tissues like the liver, pituitary, gonad, brain, adipose and adrenals ,however, the function of the enzyme in many of these tissues is poorly understood [8].

The concentration of cortisone in the body is higher than that of cortisol , cortisone also binds poorly to binding globulins, making cortisone many times more biologically available. Although cortisol is secreted by the adrenal cortex, there is a growing amount of evidence that the intracellular conversion of E to F may be an important mechanism in regulating the action of glucocorticoids [9].

It may be that 11 β-HSD type 1 allows certain tissues to convert cortisone to cortisol to increase local glucocorticoid activity and potentiate adaptive response and counteracting the type 2 activity that could result in a fall in active glucocorticoids [10]. Potentiation of the stress response would be especially important in the brain and high levels of 11 β-HSD type 1 are found around the hippocampus, further proving the role of the enzyme. 11 β-HSD type 1 also seems to play an important role in hepatocyte maturation [8].

Another emerging role of the 11 β-HSD type 1 enzyme is in the detoxification process of many non-steroidal carbonyl compounds, reduction of the carbonyl group of many toxic compounds is a common way to increase solubility and therefore increase their excretion. The 11 β-HSD type1 enzyme has recently been shown to be active in lung tissue [11]. Type 1 activity is not seen until after birth, therefore mothers who smoke during pregnancy expose their children to the harmful effects of tobacco before the child is able to metabolically detoxify this compound.

**The role of 11 β-HSD Type 2**

As already stated earlier the 11 β-HSD type 2 converts cortisol to cortisone, thus protecting the MR in many key regulatory tissues of the body. The importance of protecting the MR from occupation by glucocorticoids is seen in patients with AME or liquorice intoxification. Defects or inactivity of the type 2 enzyme results in hypertensive syndromes and research has shown that patients with an hypertensive syndrome have an increased urinary excretion ratio of cortisol : cortisone. This along with a reported increase in the half life of radiolabelled cortisol suggests a reduction of 11 β-HSD type 2 activity [12].

**Rationale for the development of 11 β-HSD inhibitors**

As said earlier cortisol opposes the action of insulin meaning a stimulation of hepatic gluconeogenesis, inhibition of peripheral glucose uptake and increased blood glucose concentration. The effects of cortisol appear to be enhanced in patients suffering from glucose intolerance or diabetes mellitus. Inhibition of the enzyme 11 β-HSD type 1 would increase glucose uptake and inhibit hepatic gluconeogenesis, giving a reduction in circulatory glucose levels. The development of a potent 11 β-HSD type 1 inhibitor could therefore have considerable therapeutic potential for conditions associated with elevated blood glucose levels.

An excess in glucocorticoids can result in neuronal dysfunctions and also impair cognitive functions. A specific 11 β-HSD type 1 inhibitor might be of some importance by reducing neuronal dysfunctions and the loss of cognitive functions associated with ageing, by blocking the conversion of cortisone to cortisol.

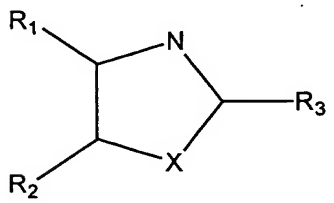
Glucocorticoids also have an important role in regulating part of the immune response [13]. Glucocorticoids can suppress the production of cytokines and regulate the receptor levels. They are also involved in determining whether T-helper (Th) lymphocytes progress into either Th1 or Th2 phenotype. These two different types of Th cells secrete a different profile of cytokines, Th2 is predominant in a glucocorticoid environment. By inhibiting 11 β-HSD type 1, Th1 cytokine response would be favoured. It is also possible to inhibit 11 β-HSD type 2 , thus by inhibiting the inactivation of cortisol, it may be possible to potentiate the anti-inflammatory effects of glucocorticoids.

Aspects of the invention are defined in the appended claims.

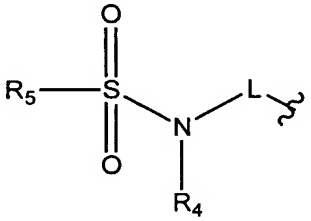
## SUMMARY ASPECTS OF THE PRESENT INVENTION

In one aspect the present invention provides a compound having Formula I

Formula I

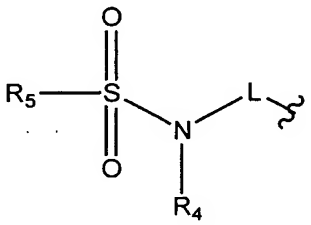



wherein one of $R_1$ and $R_2$ is a group of the formula




wherein $R_4$ is selected from H and hydrocarbyl, $R_5$ is a hydrocarbyl group and L is an optional linker group,

or $R_1$ and $R_2$ together form a ring substituted with the group



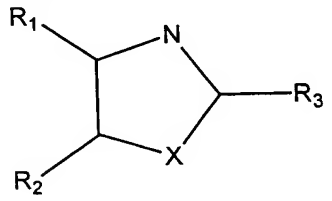

wherein $R_3$ is H or a substituent, and wherein X is selected from S, O, $NR_6$ and $C(R_7)(R_8)$, wherein $R_6$ is selected from H and hydrocarbyl groups, wherein each of $R_7$

and $R_8$ are independently selected from H and hydrocarbyl groups.

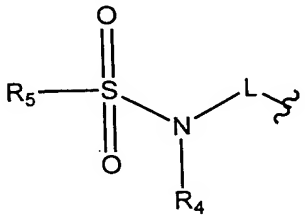
In one aspect the present invention provides a pharmaceutical composition comprising

(i) a compound having Formula I

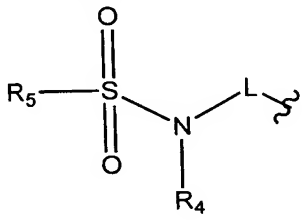
Formula I



wherein one of $R_1$ and $R_2$ is a group of the formula



wherein $R_4$ is selected from H and hydrocarbyl, $R_5$ is a hydrocarbyl group and L is an optional linker group, or $R_1$ and $R_2$ together form a ring substituted with the group

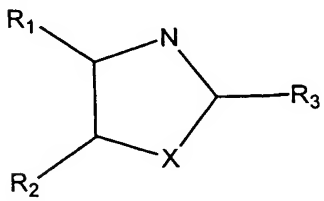


wherein $R_3$ is H or a substituent, and wherein X is selected from S, O, $NR_6$ and $C(R_7)(R_8)$, wherein $R_6$ is selected from H and hydrocarbyl groups, wherein each of $R_7$ and $R_8$ are independently selected from H and hydrocarbyl groups.

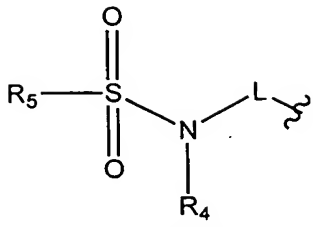
(ii) optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

In one aspect the present invention provides a compound having Formula I

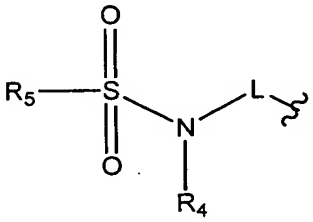
Formula I



wherein one of $R_1$ and $R_2$ is a group of the formula



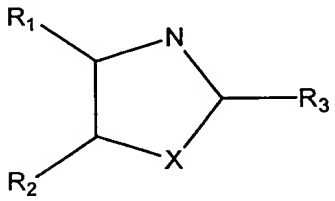
wherein $R_4$ is selected from H and hydrocarbyl, $R_5$ is a hydrocarbyl group and L is an optional linker group, or $R_1$ and $R_2$ together form a ring substituted with the group



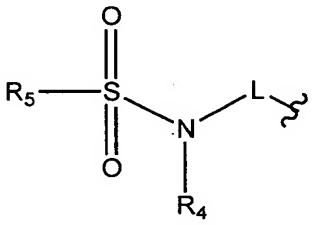
wherein $R_3$ is H or a substituent, and wherein X is selected from S, O, $NR_6$ and $C(R_7)(R_8)$, wherein $R_6$ is selected from H and hydrocarbyl groups, wherein each of $R_7$ and $R_8$ are independently selected from H and hydrocarbyl groups, for use in medicine.

In one aspect the present invention provides a use of a compound in the manufacture of a medicament for use in the therapy of a condition or disease associated with 11β-HSD, wherein the compound has Formula I

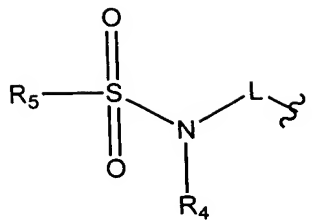
Formula I



wherein one of $R_1$ and $R_2$ is a group of the formula



wherein $R_4$ is selected from H and hydrocarbyl, $R_5$ is a hydrocarbyl group and L is an optional linker group, or $R_1$ and $R_2$ together form a ring substituted with the group



wherein $R_3$ is H or a substituent, and wherein X is selected from S, O, $NR_6$ and $C(R_7)(R_8)$, wherein $R_6$ is selected from H and hydrocarbyl groups, wherein each of $R_7$ and $R_8$ are independently selected from H and hydrocarbyl groups.

SOME ADVANTAGES

One key advantage of the present invention is that the compounds of the present invention can act as 11β-HSD inhibitors. The compounds may inhibit the interconversion of inactive 11-keto steroids with their active hydroxy equivalents. Thus present invention provides methods by which the conversion of the inactive to the active form may be controlled, and to useful therapeutic effects which may be obtained as a result of such control. More specifically, but not exclusively, the invention is concerned with interconversion between cortisone and cortisol in humans.

Another advantage of the compounds of the present invention is that they may be potent 11β-HSD inhibitors *in vivo*.

Some of the compounds of the present invention are also advantageous in that they may be orally active.

The present invention may provide for a medicament for one or more of (i) regulation of carbohydrate metabolism, (ii) regulation of protein metabolism, (iii) regulation of lipid metabolism, (iv) regulation of normal growth and/or development, (v) influence on cognitive function, (vi) resistance to stress and mineralocorticoid activity.

Some of the compounds of the present invention may also be useful for inhibiting hepatic gluconeogenesis. The present invention may also provide a medicament to relieve the effects of endogenous glucocorticoids in diabetes mellitus, obesity (including centripetal obesity), neuronal loss and/or the cognitive impairment of old age. Thus, in a further aspect, the invention provides the use of an inhibitor of 11β-HSD in the

manufacture of a medicament for producing one or more therapeutic effects in a patient to whom the medicament is administered, said therapeutic effects selected from inhibition of hepatic gluconeogenesis, an increase in insulin sensitivity in adipose tissue and muscle, and the prevention of or reduction in neuronal loss/cognitive impairment due to glucocorticoid-potentiated neurotoxicity or neural dysfunction or damage.

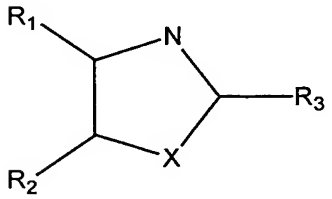
From an alternative point of view, the invention provides a method of treatment of a human or animal patient suffering from a condition selected from the group consisting of: hepatic insulin resistance, adipose tissue insulin resistance, muscle insulin resistance, neuronal loss or dysfunction due to glucocorticoid potentiated neurotoxicity, and any combination of the aforementioned conditions, the method comprising the step of administering to said patient a medicament comprising a pharmaceutically active amount of a compound in accordance with the present invention.

Some of the compounds of the present invention may be useful for the treatment of cancer, such as breast cancer, as well as (or in the alternative) non-malignant conditions, such as the prevention of auto-immune diseases, particularly when pharmaceuticals may need to be administered from an early age.

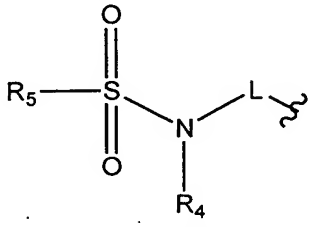
## DETAILED ASPECTS OF THE PRESENT INVENTION

In one aspect the present invention provides a compound having Formula I

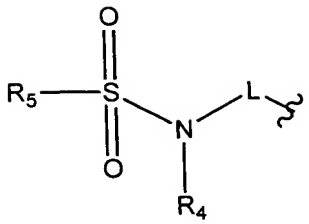
Formula I




wherein one of $R_1$ and $R_2$ is a group of the formula




wherein $R_4$ is selected from H and hydrocarbyl, $R_5$ is a hydrocarbyl group and L is an optional linker group, or $R_1$ and $R_2$ together form a ring substituted with the group



wherein $R_3$ is H or a substituent, and wherein X is selected from S, O, $NR_6$ and $C(R_7)(R_8)$, wherein $R_6$ is selected from H and hydrocarbyl groups, wherein each of $R_7$ and $R_8$ are independently selected from H and hydrocarbyl groups.

In one aspect the present invention provides a pharmaceutical composition comprising
(i) a compound having Formula I defined above
(ii) optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

In one aspect the present invention provides a compound having Formula I defined above, for use in medicine.

In one aspect the present invention provides a use of a compound having Formula I defined above in the manufacture of a medicament for use in the therapy of a condition or disease associated with 11β-HSD.

In one aspect the present invention provides a use of a compound having Formula I defined above in the manufacture of a medicament for use in the therapy of a condition or disease associated with adverse 11β-HSD levels.

In one aspect the present invention provides a use of a compound having Formula I defined above in the manufacture of a pharmaceutical for inhibiting 11β-HSD activity.

In one aspect the present invention provides a use of a compound having Formula I defined above in the manufacture of a pharmaceutical for inhibiting 11β-HSD activity.

In one aspect the present invention provides a method comprising (a) performing a 11β-HSD assay with one or more candidate compounds having Formula I defined above; (b) determining whether one or more of said candidate compounds is/are capable of modulating 11β-HSD activity; and (c) selecting one or more of said candidate

compounds that is/are capable of modulating 11β-HSD activity.

In one aspect the present invention provides a method comprising (a) performing a 11β-HSD assay with one or more candidate compounds having Formula I defined above; (b) determining whether one or more of said candidate compounds is/are capable of inhibiting 11β-HSD activity; and (c) selecting one or more of said candidate compounds that is/are capable of inhibiting 11β-HSD activity.

In one aspect the present invention provides

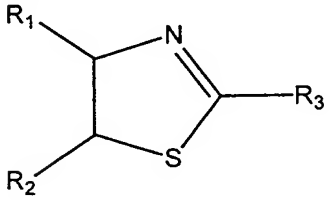
- a compound identified by the above method,
- the use of the said compound in medicine,
- a pharmaceutical composition comprising the said compound, optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant,
- use of the said compound in the manufacture of a medicament for use in the therapy of a condition or disease associated with 11β-HSD, and
- use of the said compound in the manufacture of a medicament for use in the therapy of a condition or disease associated with adverse 11β-HSD levels.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

PREFERABLE ASPECTS

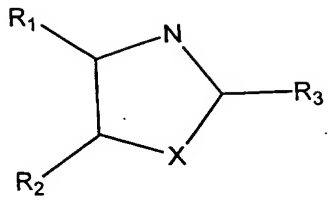
In one preferred aspect the compound of the present invention has Formula II

Formula II

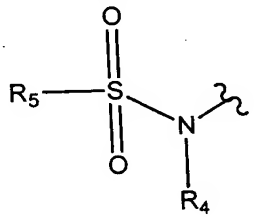



In one preferred aspect L is not present. In this aspect the present invention provides a compound having Formula I

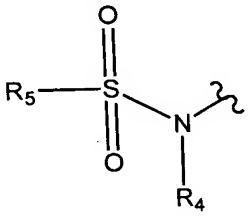
Formula I



wherein one of $R_1$ and $R_2$ is a group of the formula

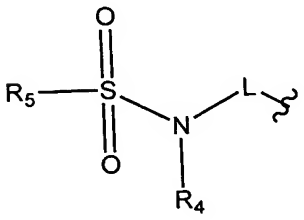


wherein $R_4$ is selected from H and hydrocarbyl, and $R_5$ is a hydrocarbyl group; or $R_1$ and $R_2$ together form a ring substituted with the group



wherein $R_3$ is H or a substituent

In one preferred aspect the compound of the present invention $R_1$ and $R_2$ together form a ring substituted with the group



In one preferred aspect the compound of the present invention $R_1$ and $R_2$ together form a carbocyclic ring.

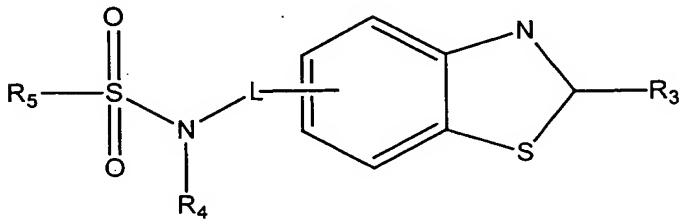
In one preferred aspect the compound of the present invention $R_1$ and $R_2$ together form a six membered ring.

In one preferred aspect the compound of the present invention $R_1$ and $R_2$ together form a six membered carbocyclic ring.

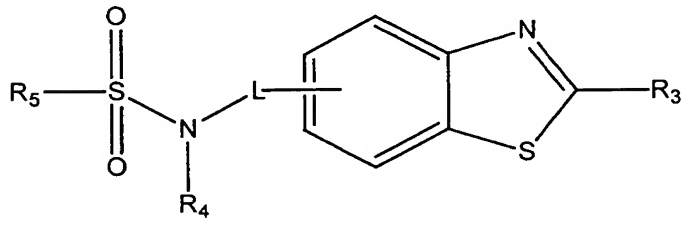
In one preferred aspect the compound of the present invention wherein $R_1$ and $R_2$ together form an aryl ring.

Preferred compounds of the present invention are those having one of the following formulae.

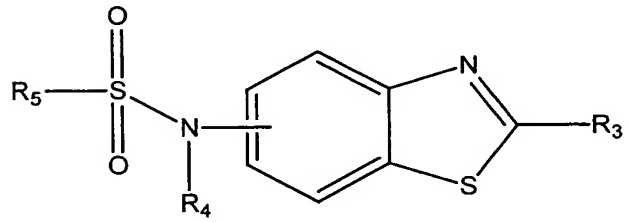
Formula III



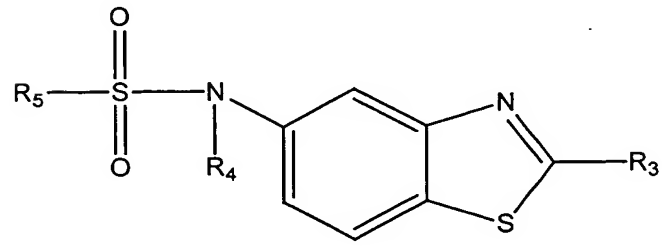
Formula IV



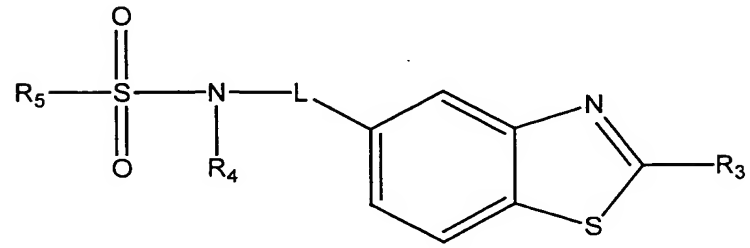
Formula V



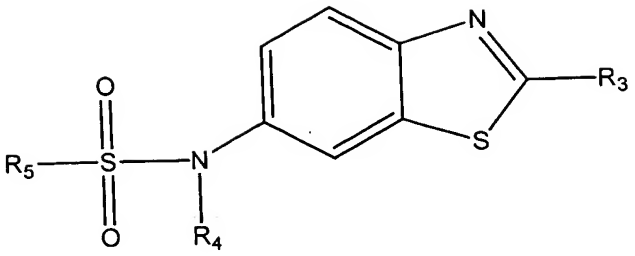
Formula VI



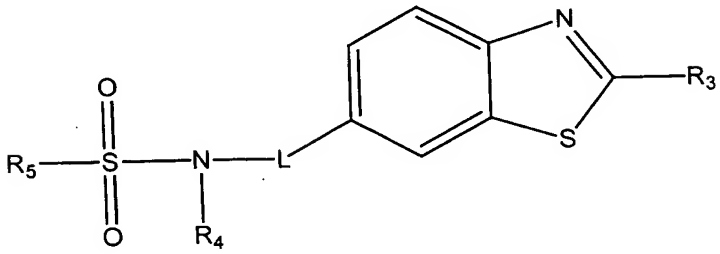
Formula VIa



Formula VII



Formula VIIa



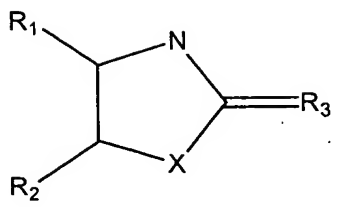
In preferred aspects of the present invention $R_3$ is selected from H, hydrocarbyl, -S-hydrocarbyl, -S-H, halogen and $N(R_9)(R_{10})$, wherein each of $R_9$ and $R_{10}$ are independently selected from H and hydrocarbyl groups.

In preferred aspects of the present invention $R_3$ is selected from H, hydroxy, alkyl especially $C_1$-$C_{10}$ alkyl groups, $C_1$-$C_6$ alkyl, e.g. $C_1$-$C_3$ alkyl group, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl and other pentyl isomers, and n-hexyl and other hexyl isomers, alkoxy especially $C_1$-$C_{10}$ alkoxy groups, $C_1$-$C_6$ alkoxy, e.g. $C_1$-$C_3$ alkoxy group, methoxy, ethoxy, propoxy etc., alkinyl, e.g. ethinyl, or halogen, e.g. fluoro substituents.

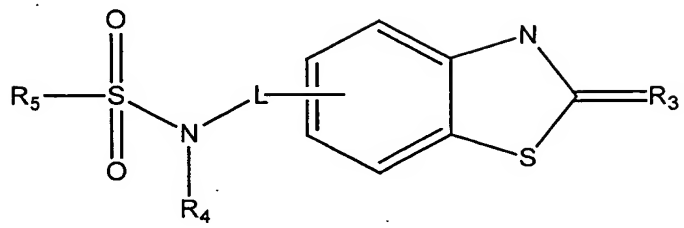
When R3 is -S-hydrocarbyl, preferably R3 is selected from –S-alkyl, -S-carboxylic acid, -S-ether, and –S-amide, preferably selected from –S-$C_{1-10}$alkyl, -S- $C_{1-10}$carboxcylic acid, -S- $C_{1-10}$ether, and –S- $C_{1-10}$amide.

In preferred aspects of the present invention $R_3$ is $-CH_3$.

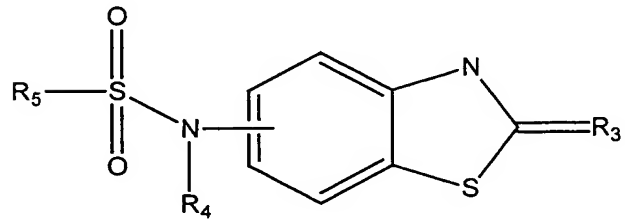
Further preferred compounds of the present invention are those having one of the following formulae.



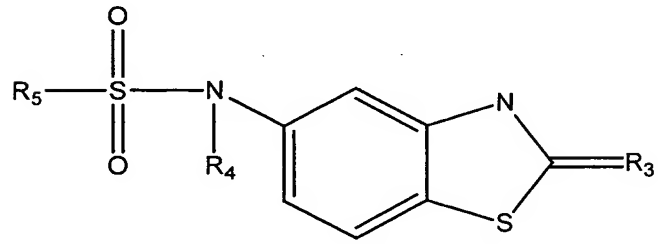
Formula Ia



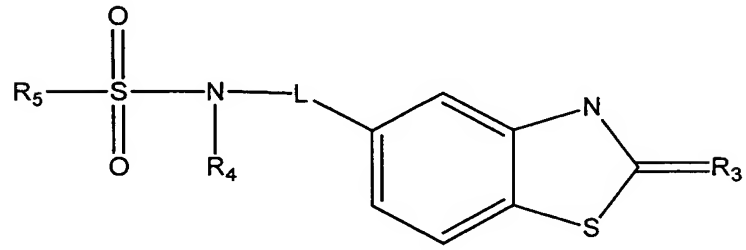
Formula VIII



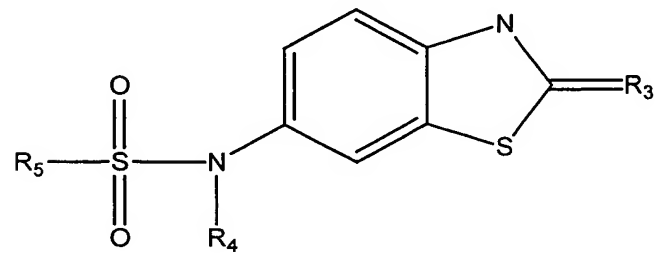
Formula IX



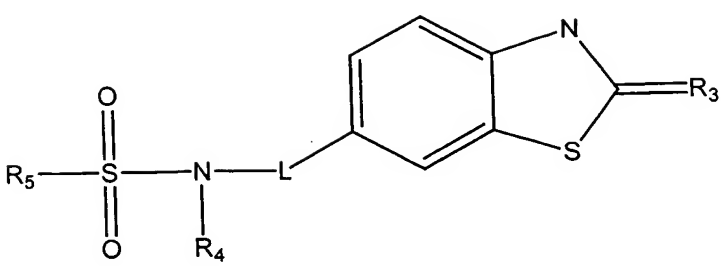
Formula X



Formula Xa



Formula XI

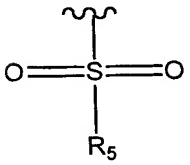


Formula XIa

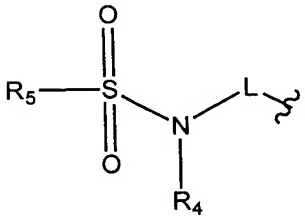
In further preferred aspects of the present invention, such as when the compound has Formula Ia, Formula VIII, Formula IX, Formula X, Formula Xa, Formula XI, or Formula XIa, $R_3$ is selected from O, hydrocarbyl, and N($R_9$) wherein $R_9$ is selected from H and hydrocarbyl groups. More preferably $R_3$ is selected from O, $C_1$-$C_{10}$ alkenyl groups, such as $C_1$-$C_6$ alkenyl group, and $C_1$-$C_3$ alkenyl group, NH and N-$C_1$-$C_{10}$ alkyl groups, such as N-$C_1$-$C_6$ alkyl group, and N-$C_1$-$C_3$ alkyl groups.

In further preferred aspects of the present invention $R_4$ is selected from H and $C_1$-$C_{10}$ alkyl groups, such as $C_1$-$C_6$ alkyl group, and $C_1$-$C_3$ alkyl group. Preferably $R_4$ is H.

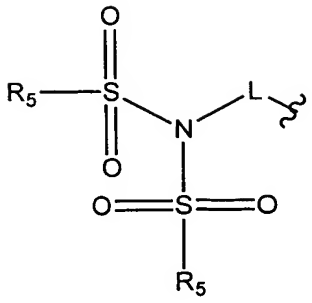
In further preferred aspects of the present invention $R_4$ is a group of the formula.



In these aspects the group shown above as



may be of the formula



wherein each $R_5$ is independently selected from hydrocarbyl groups. Each $R_5$ may be the same of different to the other $R_5$. In one aspect the two $R_5$ groups are the same.

In some preferred aspects of the invention $R_5$ is a cyclic hydrocarbyl group. Preferably $R_5$ is a cyclic hydrocarbyl group comprising a hydrocarbon ring.

$R_5$ may be a substituted ring or an unsubstituted ring. In some preferred aspects of the invention $R_5$ is substituted ring.

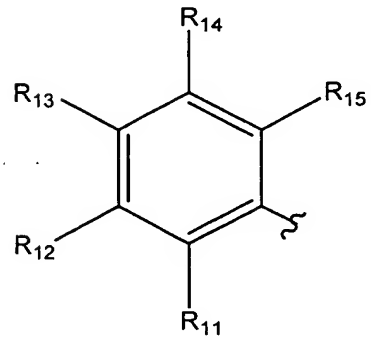
Preferably $R_5$ is a carbocyclic ring.

Preferably $R_5$ is a six membered ring.

Preferably $R_5$ is a six membered carbocyclic ring. More preferably $R_5$ is a substituted six membered carbocyclic ring.

In some preferred aspects of the invention $R_5$ is an aryl ring. Preferably $R_5$ is a substituted aryl ring.

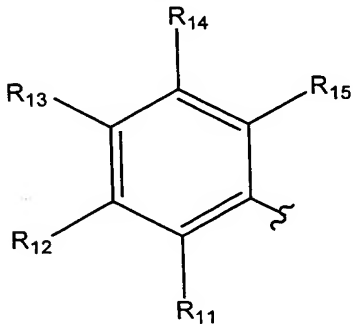
In one highly preferred aspect $R_5$ is a group having the formula



wherein each of $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ are independently selected from H, halogen, and hydrocarbyl groups.

Preferably each of $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ are independently selected from H, halogen, alkyl, such as $C_{1-6}$ alkyl, phenyl, O-alkyl, O-phenyl, nitrile, haloalkyl, such as $CF_3$, $CCl_3$ and $CBr_3$, carboxyalkyl, $-CO_2H$, $CO_2$alkyl, and NH-acetyl groups..

Two or more of $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ may join to form a ring. The two or more of $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ may or may not be adjacent. The ring may be carbocyclic or heterocyclic ring. The ring may be optionally substituted by any of the $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ substituents listed above. When two or more of $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ may join to form a ring the group




may provide a naphthyl, quinolyl, tetrahydroquinolyl, or benzothtrahydropyranyl, each of which may be substituted or unsubstituted.

SUBSTITUENTS

The compound of the present invention may have substituents other than those of the ring systems show herein. Furthermore the ring systems herein are given as general formulae and should be interpreted as such. The absence of any specifically shown substituents on a given ring member indicates that the ring member may substituted with any moiety of which H is only one example. The ring system may contain one or more degrees of unsaturation, for example is some aspects one or more rings of the ring system is aromatic. The ring system may be carbocyclic or may contain one or more hetero atoms.

The compound of the invention, in particular the ring system compound of the invention of the present invention may contain substituents other than those show herein. By way

of example, these other substituents may be one or more of: one or more halo groups, one or more O groups, one or more hydroxy groups, one or more amino groups, one or more sulphur containing group(s), one or more hydrocarbyl group(s) – such as an oxyhydrocarbyl group.

In general terms the ring system of the present compounds may contain a variety of non-interfering substituents. In particular, the ring system may contain one or more hydroxy, alkyl especially lower ($C_1$-$C_6$) alkyl, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl and other pentyl isomers, and n-hexyl and other hexyl isomers, alkoxy especially lower ($C_1$-$C_6$) alkoxy, e.g. methoxy, ethoxy, propoxy etc., alkinyl, e.g. ethinyl, or halogen, e.g. fluoro substituents.

For some compounds of the present invention, the compound may be substituted with a hydrocarbylsulphanyl group. The term "hydrocarbylsulphanyl" means a group that comprises at least hydrocarbyl group (as herein defined) and sulphur, preferably –S-hydrocarbyl, more preferably –S-hydrocarbon. That sulphur group may be optionally oxidised.

Preferably the hydrocarbylsulphanyl group is –S-$C_{1-10}$ alkyl, more preferably –S-$C_{1-5}$ alkyl, more preferably –S-$C_{1-3}$ alkyl, more preferably –S-$CH_2CH_2CH_3$, –S-$CH_2CH_3$ or –$SCH_3$

FURTHER ASPECTS

For some applications, preferably the compounds have a reversible action.

For some applications, preferably the compounds have an irreversible action.

In one embodiment, the compounds of the present invention are useful for the treatment of breast cancer.

The compounds of the present invention may be in the form of a salt.

The present invention also covers novel intermediates that are useful to prepare the compounds of the present invention. For example, the present invention covers novel

alcohol precursors for the compounds. By way of further example, the present invention covers bis protected precursors for the compounds. Examples of each of these precursors are presented herein. The present invention also encompasses a process comprising each or both of those precursors for the synthesis of the compounds of the present invention.

STEROID DEHYDROGENASE

11ß Steroid dehydrogenase may be referred to as "11ß-HSD" or "HD" for short

In some aspects of the invention 11β-HSD is preferably 11β-HSD Type 1.

In some aspects of the invention 11β-HSD is preferably 11β-HSD Type 2.

STEROID DEHYDROGENASE INHIBITION

It is believed that some disease conditions associated with DH activity are due to conversion of a inactive, oestrone to an active, oestradiol. In disease conditions associated with DH activity, it would be desirable to inhibit DH activity.

Here, the term "inhibit" includes reduce and/or eliminate and/or mask and/or prevent the detrimental action of DH.

DH INHIBITOR

In accordance with the present invention, the compound of the present invention is capable of acting as an DH inhibitor.

Here, the term "inhibitor" as used herein with respect to the compound of the present invention means a compound that can inhibit DH activity – such as reduce and/or eliminate and/or mask and/or prevent the detrimental action of DH. The DH inhibitor may act as an antagonist.

The ability of compounds to inhibit steroid dehydrogenase activity can be assessed using the suitable Assay Protocol presented in the Examples section.

It is to be noted that the compound of the present invention may have other beneficial properties in addition to or in the alternative to its ability to inhibit DH activity.

HYDROCARBYL

The term "hydrocarbyl group" as used herein means a group comprising at least C and H and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo, alkoxy, nitro, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the hydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked via a suitable element or group. Thus, the hydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur, nitrogen and oxygen. A non-limiting example of a hydrocarbyl group is an acyl group.

A typical hydrocarbyl group is a hydrocarbon group. Here the term "hydrocarbon" means any one of an alkyl group, an alkenyl group, an alkynyl group, which groups may be linear, branched or cyclic, or an aryl group. The term hydrocarbon also includes those groups but wherein they have been optionally substituted. If the hydrocarbon is a branched structure having substituent(s) thereon, then the substitution may be on either the hydrocarbon backbone or on the branch; alternatively the substitutions may be on the hydrocarbon backbone and on the branch.

In some aspects of the present invention, one or more hydrocarbyl groups is independently selected from optionally substituted alkyl group, optionally substituted haloalkyl group, aryl group, alkylaryl group, alkylarylakyl group, and an alkene group.

In some aspects of the present invention, one or more hydrocarbyl groups is independently selected from $C_1$-$C_{10}$ alkyl group, such as $C_1$-$C_6$ alkyl group, and $C_1$-$C_3$ alkyl group. Typical alkyl groups include $C_1$ alkyl, $C_2$ alkyl, $C_3$ alkyl, $C_4$ alkyl, $C_5$ alkyl, $C_7$ alkyl, and $C_8$ alkyl.

In some aspects of the present invention, one or more hydrocarbyl groups is

independently selected from $C_1$-$C_{10}$ haloalkyl group, $C_1$-$C_6$ haloalkyl group, $C_1$-$C_3$ haloalkyl group, $C_1$-$C_{10}$ bromoalkyl group, $C_1$-$C_6$ bromoalkyl group, and $C_1$-$C_3$ bromoalkyl group. Typical haloalkyl groups include $C_1$ haloalkyl, $C_2$ haloalkyl, $C_3$ haloalkyl, $C_4$ haloalkyl, $C_5$ haloalkyl, $C_7$ haloalkyl, $C_8$ haloalkyl, $C_1$ bromoalkyl, $C_2$ bromoalkyl, $C_3$ bromoalkyl, $C_4$ bromoalkyl, $C_5$ bromoalkyl, $C_7$ bromoalkyl, and $C_8$ bromoalkyl.

In some aspects of the present invention, one or more hydrocarbyl groups is independently selected from aryl groups, alkylaryl groups, alkylarylakyl groups, -$(CH_2)_{1-10}$-aryl, -$(CH_2)_{1-10}$-Ph, $(CH_2)_{1-10}$-Ph-$C_{1-10}$ alkyl, -$(CH_2)_{1-5}$-Ph, $(CH_2)_{1-5}$-Ph-$C_{1-5}$ alkyl, -$(CH_2)_{1-3}$-Ph, $(CH_2)_{1-3}$-Ph-$C_{1-3}$ alkyl, -$CH_2$-Ph, and -$CH_2$-Ph-$C(CH_3)_3$. The aryl groups may contain a hetero atom. Thus the aryl group or one or more of the aryl groups may be carbocyclic or more may heterocyclic. Typical hetero atoms include O, N and S, in particular N.

In some aspects of the present invention, one or more hydrocarbyl groups is independently selected from -$(CH_2)_{1-10}$-cycloalkyl, -$(CH_2)_{1-10}$-$C_{3-10}$cycloalkyl, -$(CH_2)_{1-7}$-$C_{3-7}$cycloalkyl, -$(CH_2)_{1-5}$-$C_{3-5}$cycloalkyl, -$(CH_2)_{1-3}$-$C_{3-5}$cycloalkyl, and -$CH_2$- $C_3$cycloalkyl.

In some aspects of the present invention, one or more hydrocarbyl groups is independently selected from alkene groups. Typical alkene groups include $C_1$-$C_{10}$ alkene group, $C_1$-$C_6$ alkene group, $C_1$-$C_3$ alkene group, such as $C_1$, $C_2$, $C_3$, $C_4$, $C_5$, $C_6$, or $C_7$ alkene group. In a preferred aspect the alkene group contains 1, 2 or 3 C=C bonds. In a preferred aspect the alkene group contains 1 C=C bond. In some preferred aspect at least one C=C bond or the only C=C bond is to the terminal C of the alkene chain, that is the bond is at the distal end of the chain to the ring system.

In some aspects of the present invention, one or more hydrocarbyl groups is independently selected from oxyhydrocarbyl groups.

OXYHYDROCARBYL

The term "oxyhydrocarbyl" group as used herein means a group comprising at least C, H and O and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo-, alkoxy-, nitro-, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of

substituents may form a cyclic group. If the oxyhydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the oxyhydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur and nitrogen.

In one embodiment of the present invention, the oxyhydrocarbyl group is a oxyhydrocarbon group.

Here the term "oxyhydrocarbon" means any one of an alkoxy group, an oxyalkenyl group, an oxyalkynyl group, which groups may be linear, branched or cyclic, or an oxyaryl group. The term oxyhydrocarbon also includes those groups but wherein they have been optionally substituted. If the oxyhydrocarbon is a branched structure having substituent(s) thereon, then the substitution may be on either the hydrocarbon backbone or on the branch; alternatively the substitutions may be on the hydrocarbon backbone and on the branch.

Typically, the oxyhydrocarbyl group is of the formula $C_{1-6}O$ (such as a $C_{1-3}O$).

## ANIMAL ASSAY MODEL FOR DETERMINING OESTROGENIC ACTIVITY (PROTOCOL 1)

Lack of *in vivo* oestrogenicity

The compounds of the present invention may be studied using an animal model, in particular in ovariectomised rats. In this model, compounds which are oestrogenic stimulate uterine growth.

The compound (10 mg/Kg/day for five days) was administered orally to rats with another group of animals receiving vehicle only (propylene glycol). A further group received the estrogenic compound EMATE subcutaneously in an amount of 10µg/day for five days. At the end of the study uteri were obtained and weighed with the results being expressed as uterine weight/whole body weight x 100.

Compounds having no significant effect on uterine growth are not oestrogenic.

REPORTERS

A wide variety of reporters may be used in the assay methods (as well as screens) of the present invention with preferred reporters providing conveniently detectable signals (e.g. by spectroscopy). By way of example, a reporter gene may encode an enzyme which catalyses a reaction which alters light absorption properties.

Other protocols include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes may even be used. These and other assays are described, among other places, in Hampton R et al (1990, Serological Methods, A Laboratory Manual, APS Press, St Paul MN) and Maddox DE et al (1983, J Exp Med 15 8:121 1).

Examples of reporter molecules include but are not limited to (β-galactosidase, invertase, green fluorescent protein, luciferase, chloramphenicol, acetyltransferase, (-glucuronidase, exo-glucanase and glucoamylase. Alternatively, radiolabelled or fluorescent tag-labelled nucleotides can be incorporated into nascent transcripts which are then identified when bound to oligonucleotide probes.

By way of further examples, a number of companies such as Pharmacia Biotech (Piscataway, NJ), Promega (Madison, WI), and US Biochemical Corp (Cleveland, OH) supply commercial kits and protocols for assay procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US-A-3817837; US-A-3850752; US-A-3939350; US-A-3996345; US-A-4277437; US-A-4275149 and US-A-4366241.

HOST CELLS

The term "host cell" - in relation to the present invention includes any cell that could comprise the target for the agent of the present invention.

Thus, a further embodiment of the present invention provides host cells transformed or

transfected with a polynucleotide that is or expresses the target of the present invention. Preferably said polynucleotide is carried in a vector for the replication and expression of polynucleotides that are to be the target or are to express the target. The cells will be chosen to be compatible with the said vector and may for example be prokaryotic (for example bacterial), fungal, yeast or plant cells.

The gram negative bacterium *E. coli* is widely used as a host for heterologous gene expression. However, large amounts of heterologous protein tend to accumulate inside the cell. Subsequent purification of the desired protein from the bulk of *E.coli* intracellular proteins can sometimes be difficult.

In contrast to *E.coli*, bacteria from the genus *Bacillus* are very suitable as heterologous hosts because of their capability to secrete proteins into the culture medium. Other bacteria suitable as hosts are those from the genera Streptomyces and Pseudomonas.

Depending on the nature of the polynucleotide encoding the polypeptide of the present invention, and/or the desirability for further processing of the expressed protein, eukaryotic hosts such as yeasts or other fungi may be preferred. In general, yeast cells are preferred over fungal cells because they are easier to manipulate. However, some proteins are either poorly secreted from the yeast cell, or in some cases are not processed properly (e.g. hyperglycosylation in yeast). In these instances, a different fungal host organism should be selected.

Examples of suitable expression hosts within the scope of the present invention are fungi such as Aspergillus species (such as those described in EP-A-0184438 and EP-A-0284603) and Trichoderma species; bacteria such as Bacillus species (such as those described in EP-A-0134048 and EP-A-0253455), Streptomyces species and Pseudomonas species; and yeasts such as Kluyveromyces species (such as those described in EP-A-0096430 and EP-A-0301670) and Saccharomyces species. By way of example, typical expression hosts may be selected from *Aspergillus niger*, *Aspergillus niger var. tubigenis*, *Aspergillus niger var. awamori*, *Aspergillus aculeatis*, *Aspergillus nidulans*, *Aspergillus orvzae*, *Trichoderma reesei*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Kluyveromyces lactis* and *Saccharomyces cerevisiae*.

The use of suitable host cells - such as yeast, fungal and plant host cells - may provide

for post-translational modifications (e.g. myristoylation, glycosylation, truncation, lapidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the present invention.

ORGANISM

The term "organism" in relation to the present invention includes any organism that could comprise the target according to the present invention and/or products obtained therefrom. Examples of organisms may include a fungus, yeast or a plant.

The term "transgenic organism" in relation to the present invention includes any organism that comprises the target according to the present invention and/or products obtained.

TRANSFORMATION OF HOST CELLS/HOST ORGANISMS

As indicated earlier, the host organism can be a prokaryotic or a eukaryotic organism. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis.* Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press) and Ausubel *et al.*, Current Protocols in Molecular Biology (1995), John Wiley & Sons, Inc.

If a prokaryotic host is used then the nucleotide sequence may need to be suitably modified before transformation - such as by removal of introns.

In another embodiment the transgenic organism can be a yeast. In this regard, yeast have also been widely used as a vehicle for heterologous gene expression. The species Saccharomyces cerevisiae has a long history of industrial use, including its use for heterologous gene expression. Expression of heterologous genes in *Saccharomyces cerevisiae* has been reviewed by Goodey *et al* (1987, Yeast Biotechnology, D R Berry *et al*, eds, pp 401-429, Allen and Unwin, London) and by King *et al* (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and it is incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of knowledge about the genetics and physiology as well as large-scale fermentation characteristics of Saccharomyces cerevisiae.

A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating plasmid vectors.

In order to prepare the transgenic Saccharomyces, expression constructs are prepared by inserting the nucleotide sequence into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been developed. The constructs contain a promoter active in yeast fused to the nucleotide sequence, usually a promoter of yeast origin, such as the GAL1 promoter, is used. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

For the transformation of yeast several transformation protocols have been developed. For example, a transgenic Saccharomyces according to the present invention can be prepared by following the teachings of Hinnen et al (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929); Beggs, J D (1978, Nature, London, 275, 104); and Ito, H et al (1983, J Bacteriology 153, 163-168).

The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside antibiotic markers, e.g. G418.

Another host organism is a plant. The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material. Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). Further teachings on plant transformation may be found in EP-A-0449375.

Thus, the present invention also provides a method of transforming a host cell with a nucleotide sequence that is to be the target or is to express the target. Host cells transformed with the nucleotide sequence may be cultured under conditions suitable for the expression of the encoded protein. The protein produced by a recombinant cell may be displayed on the surface of the cell. If desired, and as will be understood by those of skill in the art, expression vectors containing coding sequences can be designed with signal sequences which direct secretion of the coding sequences through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join the coding sequence to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll DJ et al (1993) DNA Cell Biol 12:441-53).

VARIANTS/HOMOLOGUES/DERIVATIVES

In addition to the specific amino acid sequences and nucleotide sequences mentioned herein, the present invention also encompasses the use of variants, homologue and derivatives thereof. Here, the term "homology" can be equated with "identity".

In the present context, an homologous sequence is taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily

available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux et al., 1984, Nucleic Acids Research 12:387). Examples of

other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 ibid – Chapter 18), FASTA (Atschul et al., 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999 ibid, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program.

A further useful reference is that found in FEMS Microbiol Lett 1999 May 15;174(2):247-50 (and a published erratum appears in FEMS Microbiol Lett 1999 Aug 1;177(1):187-8).

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below.

Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

| | | |
|---|---|---|
| ALIPHATIC | Non-polar | G A P |
| | | I L V |
| | Polar – uncharged | C S T M |
| | | N Q |
| | Polar – charged | D E |
| | | K R |
| AROMATIC | | H F W Y |

EXPRESSION VECTORS

The nucleotide sequence for use as the target or for expressing the target can be incorporated into a recombinant replicable vector. The vector may be used to replicate and express the nucleotide sequence in and/or from a compatible host cell. Expression may be controlled using control sequences which include promoters/enhancers and other expression regulation signals. Prokaryotic promoters and promoters functional in eukaryotic cells may be used. Tissue specific or stimuli specific promoters may be used. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

The protein produced by a host recombinant cell by expression of the nucleotide sequence may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. The coding sequences can be designed with signal sequences which direct secretion of the substance coding sequences through a particular prokaryotic or eukaryotic cell membrane.

FUSION PROTEINS

The target amino acid sequence may be produced as a fusion protein, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and (-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of

interest to allow removal of fusion protein sequences. Preferably the fusion protein will not hinder the activity of the target.

The fusion protein may comprise an antigen or an antigenic determinant fused to the substance of the present invention. In this embodiment, the fusion protein may be a non-naturally occurring fusion protein comprising a substance which may act as an adjuvant in the sense of providing a generalised stimulation of the immune system. The antigen or antigenic determinant may be attached to either the amino or carboxy terminus of the substance.

In another embodiment of the invention, the amino acid sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for agents capable of affecting the substance activity, it may be useful to encode a chimeric substance expressing a heterologous epitope that is recognised by a commercially available antibody.

THERAPY

The compounds of the present invention may be used as therapeutic agents – i.e. in therapy applications.

The term "therapy" includes curative effects, alleviation effects, and prophylactic effects.

The therapy may be on humans or animals, preferably female animals.

PHARMACEUTICAL COMPOSITIONS

In one aspect, the present invention provides a pharmaceutical composition, which comprises a compound according to the present invention and optionally a pharmaceutically acceptable carrier, diluent or excipient (including combinations thereof).

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, or excipient. Acceptable carriers or diluents for therapeutic

use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

Preservatives, stabilisers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestable solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

Where the agent is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the

solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

COMBINATION PHARMACEUTICAL

The compound of the present invention may be used in combination with one or more other active agents, such as one or more other pharmaceutically active agents.

By way of example, the compounds of the present invention may be used in combination with other 11β-HSD inhibitors and/or other inhibitors such as an aromatase inhibitor (such as for example, 4hydroxyandrostenedione (4-OHA)), and/or a steroid sulphatase inhibitors such as EMATE and/or steroids – such as the naturally occurring sterneurosteroids dehydroepiandrosterone sulfate (DHEAS) and pregnenolone sulfate (PS) and/or other structurally similar organic compounds.

In addition, or in the alternative, the compound of the present invention may be used in combination with a biological response modifier.

The term biological response modifier ("BRM") includes cytokines, immune modulators, growth factors, haematopoiesis regulating factors, colony stimulating factors, chemotactic, haemolytic and thrombolytic factors, cell surface receptors, ligands, leukocyte adhesion molecules, monoclonal antibodies, preventative and therapeutic vaccines, hormones, extracellular matrix components, fibronectin, etc. For some applications, preferably, the biological response modifier is a cytokine. Examples of cytokines include: interleukins (IL) - such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-19; Tumour Necrosis Factor (TNF) - such as TNF-α; Interferon alpha, beta and gamma; TGF-β. For some applications, preferably the cytokine is tumour necrosis factor (TNF). For some applications, the TNF may be any type of TNF - such as TNF-α, TNF-β, including derivatives or mixtures thereof. More preferably the cytokine is TNF-α. Teachings on TNF may be found in the art - such as WO-A-98/08870 and WO-A-98/13348.

ADMINISTRATION

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The dosages below are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration. Depending upon the need, the agent may be administered at a dose of from 0.01 to 30 mg/kg body weight, such as from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

By way of further example, the agents of the present invention may be administered in accordance with a regimen of 1 to 4 times per day, preferably once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

Aside from the typical modes of delivery – indicated above – the term "administered" also includes delivery by techniques such as lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

The term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

Thus, for pharmaceutical administration, the compounds of the present invention can be formulated in any suitable manner utilising conventional pharmaceutical formulating techniques and pharmaceutical carriers, adjuvants, excipients, diluents etc. and usually

for parenteral administration. Approximate effective dose rates may be in the range from 1 to 1000 mg/day, such as from 10 to 900 mg/day or even from 100 to 800 mg/day depending on the individual activities of the compounds in question and for a patient of average (70Kg) bodyweight. More usual dosage rates for the preferred and more active compounds will be in the range 200 to 800 mg/day, more preferably, 200 to 500 mg/day, most preferably from 200 to 250 mg/day. They may be given in single dose regimes, split dose regimes and/or in multiple dose regimes lasting over several days. For oral administration they may be formulated in tablets, capsules, solution or suspension containing from 100 to 500 mg of compound per unit dose. Alternatively and preferably the compounds will be formulated for parenteral administration in a suitable parenterally administrable carrier and providing single daily dosage rates in the range 200 to 800 mg, preferably 200 to 500, more preferably 200 to 250 mg. Such effective daily doses will, however, vary depending on inherent activity of the active ingredient and on the bodyweight of the patient, such variations being within the skill and judgement of the physician.

CELL CYCLING

The compounds of the present invention may be useful in the method of treatment of a cell cycling disorder.

As discussed in "Molecular Cell Biology" 3rd Ed. Lodish *et al.* pages 177-181 different eukaryotic cells can grow and divide at quite different rates. Yeast cells, for example, can divide every 120 min., and the first divisions of fertilised eggs in the embryonic cells of sea urchins and insects take only 1530 min. because one large pre-existing cell is subdivided. However, most growing plant and animal cells take 10-20 hours to double in number, and some duplicate at a much slower rate. Many cells in adults, such as nerve cells and striated muscle cells, do not divide at all; others, like the fibroblasts that assist in healing wounds, grow on demand but are otherwise quiescent.

Still, every eukaryotic cell that divides must be ready to donate equal genetic material to two daughter cells. DNA synthesis in eukaryotes does not occur throughout the cell division cycle but is restricted to a part of it before cell division.

The relationship between eukaryotic DNA synthesis and cell division has been

thoroughly analysed in cultures of mammalian cells that were all capable of growth and division. In contrast to bacteria, it was found, eukaryotic cells spend only a part of their time in DNA synthesis, and it is completed hours before cell division (mitosis). Thus a gap of time occurs after DNA synthesis and before cell division; another gap was found to occur after division and before the next round of DNA synthesis. This analysis led to the conclusion that the eukaryotic cell cycle consists of an M (mitotic) phase, a $G_1$ phase (the first gap), the S (DNA synthesis) phase, a $G_2$ phase (the second gap), and back to M. The phases between mitoses ($G_1$, S, and $G_2$) are known collectively as the interphase.

Many nondividing cells in tissues (for example, all quiescent fibroblasts) suspend the cycle after mitosis and just prior to DNA synthesis; such "resting" cells are said to have exited from the cell cycle and to be in the $G_0$ state.

It is possible to identify cells when they are in one of the three interphase stages of the cell cycle, by using a fluorescence-activated cell sorter (FACS) to measure their relative DNA content: a cell that is in $G_1$ (before DNA synthesis) has a defined amount $x$ of DNA; during S (DNA replication), it has between $x$ and $2x$; and when in $G_2$ (or M), it has $2x$ of DNA.

The stages of mitosis and cytokinesis in an animal cell are as follows

(a) Interphase. The $G_2$ stage of interphase immediately precedes the beginning of mitosis. Chromosomal DNA has been replicated and bound to protein during the S phase, but chromosomes are not yet seen as distinct structures. The nucleolus is the only nuclear substructure that is visible under light microscope. In a diploid cell before DNA replication there are two morphologic chromosomes of each type, and the cell is said to be 2n. In $G_2$, after DNA replication, the cell is 4n. There are four copies of each chromosomal DNA. Since the sister chromosomes have not yet separated from each other, they are called sister chromatids.

b) Early prophase. Centrioles, each with a newly formed daughter centriole, begin moving toward opposite poles of the cell; the chromosomes can be seen as long threads. The nuclear membrane begins to disaggregate into small vesicles.

(c) Middle and late prophase. Chromosome condensation is completed; each visible chromosome structure is composed of two chromatids held together at their centromeres. Each chromatid contains one of the two newly replicated daughter DNA molecules. The microtubular spindle begins to radiate from the regions just adjacent to the centrioles, which are moving closer to their poles. Some spindle fibres reach from pole to pole; most go to chromatids and attach at kinetochores.

(d) Metaphase. The chromosomes move toward the equator of the cell, where they become aligned in the equatorial plane. The sister chromatids have not yet separated.

(e) Anaphase. The two sister chromatids separate into independent chromosomes. Each contains a centromere that is linked by a spindle fibre to one pole, to which it moves. Thus one copy of each chromosome is donated to each daughter cell. Simultaneously, the cell elongates, as do the pole-to-pole spindles. Cytokinesis begins as the cleavage furrow starts to form.

(f) Telophase. New membranes form around the daughter nuclei; the chromosomes uncoil and become less distinct, the nucleolus becomes visible again, and the nuclear membrane forms around each daughter nucleus. Cytokinesis is nearly complete, and the spindle disappears as the microtubules and other fibres depolymerise. Throughout mitosis the "daughter" centriole at each pole grows until it is full-length. At telophase the duplication of each of the original centrioles is completed, and new daughter centrioles will be generated during the next interphase.

(g) Interphase. Upon the completion of cytokinesis, the cell enters the $G_1$ phase of the cell cycle and proceeds again around the cycle.

It will be appreciated that cell cycling is an extremely important cell process. Deviations from normal cell cycling can result in a number of medical disorders. Increased and/or unrestricted cell cycling may result in cancer. Reduced cell cycling may result in degenerative conditions. Use of the compound of the present invention may provide a means to treat such disorders and conditions.

Thus, the compound of the present invention may be suitable for use in the treatment of cell cycling disorders such as cancers, including hormone dependent and hormone

independent cancers.

In addition, the compound of the present invention may be suitable for the treatment of cancers such as breast cancer, ovarian cancer, endometrial cancer, sarcomas, melanomas, prostate cancer, pancreatic cancer etc. and other solid tumours.

For some applications, cell cycling is inhibited and/or prevented and/or arrested, preferably wherein cell cycling is prevented and/or arrested. In one aspect cell cycling may be inhibited and/or prevented and/or arrested in the $G_2$/M phase. In one aspect cell cycling may be irreversibly prevented and/or inhibited and/or arrested, preferably wherein cell cycling is irreversibly prevented and/or arrested.

By the term "irreversibly prevented and/or inhibited and/or arrested" it is meant after application of a compound of the present invention, on removal of the compound the effects of the compound, namely prevention and/or inhibition and/or arrest of cell cycling, are still observable. More particularly by the term "irreversibly prevented and/or inhibited and/or arrested" it is meant that when assayed in accordance with the cell cycling assay protocol presented herein, cells treated with a compound of interest show less growth after Stage 2 of the protocol I than control cells. Details on this protocol are presented below.

Thus, the present invention provides compounds which: cause inhibition of growth of oestrogen receptor positive (ER+) and ER negative (ER-) breast cancer cells *in vitro* by preventing and/or inhibiting and/or arresting cell cycling; and/or cause regression of nitroso-methyl urea (NMU)-induced mammary tumours in intact animals (i.e. not ovariectomised), and/or prevent and/or inhibit and/or arrest cell cycling in cancer cells; and/or act *in vivo* by preventing and/or inhibiting and/or arresting cell cycling and/or act as a cell cycling agonist.

## CELL CYCLING ASSAY (PROTOCOL 2)

Procedure

Stage 1

MCF-7 breast cancer cells are seeded into multi-well culture plates at a density of 105 cells/well. Cells were allowed to attach and grown until about 30% confluent when they

are treated as follows:

Control - no treatment
Compound of Interest (COI) 20μM

Cells are grown for 6 days in growth medium containing the COI with changes of medium/COI every 3 days. At the end of this period cell numbers were counted using a Coulter cell counter.

Stage 2

After treatment of cells for a 6-day period with the COI cells are re-seeded at a density of $10^4$ cells/well. No further treatments are added. Cells are allowed to continue to grow for a further 6 days in the presence of growth medium. At the end of this period cell numbers are again counted.

CANCER

As indicated, the compounds of the present invention may be useful in the treatment of a cell cycling disorder. A particular cell cycling disorder is cancer.

Cancer remains a major cause of mortality in most Western countries. Cancer therapies developed so far have included blocking the action or synthesis of hormones to inhibit the growth of hormone-dependent tumours. However, more aggressive chemotherapy is currently employed for the treatment of hormone-independent tumours.

Hence, the development of a pharmaceutical for anti-cancer treatment of hormone dependent and/or hormone independent tumours, yet lacking some or all of the side-effects associated with chemotherapy, would represent a major therapeutic advance.

We believe that the compound of the present invention provides a means for the treatment of cancers and, especially, breast cancer.

In addition or in the alternative the compound of the present invention may be useful in the blocking the growth of cancers including leukaemias and solid tumours such as

breast, endometrium, prostate, ovary and pancreatic tumours.

OTHER THERAPIES

It is also to be understood that the compound/composition of the present invention may have other important medical implications.

For example, the compound or composition of the present invention may be useful in the treatment of the disorders listed in WO-A-99/52890 – *viz*:

In addition, or in the alternative, the compound or composition of the present invention may be useful in the treatment of the disorders listed in WO-A-98/05635. For ease of reference, part of that list is now provided: diabetes including Type II diabetes, obesity, cancer, inflammation or inflammatory disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia, anorexia, acute infection, HIV infection, shock states, graft-versus-host reactions, autoimmune disease, reperfusion injury, meningitis, migraine and aspirin-dependent anti-thrombosis; tumour growth, invasion and spread, angiogenesis, metastases, malignant, ascites and malignant pleural effusion; cerebral ischaemia, ischaemic heart disease, osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis; periodontitis, gingivitis; psoriasis, atopic dermatitis, chronic ulcers, epidermolysis bullosa; corneal ulceration, retinopathy and surgical wound healing; rhinitis, allergic conjunctivitis, eczema, anaphylaxis; restenosis, congestive heart failure, endometriosis, atherosclerosis or endosclerosis.

In addition, or in the alternative, the compound or composition of the present invention may be useful in the treatment of disorders listed in WO-A-98/07859. For ease of reference, part of that list is now provided: cytokine and cell proliferation/differentiation activity; immunosuppressant or immunostimulant activity (e.g. for treating immune deficiency, including infection with human immune deficiency virus; regulation of lymphocyte growth; treating cancer and many autoimmune diseases, and to prevent transplant rejection or induce tumour immunity); regulation of haematopoiesis, e.g. treatment of myeloid or lymphoid diseases; promoting growth of bone, cartilage, tendon, ligament and nerve tissue, e.g. for healing wounds, treatment of burns, ulcers and

periodontal disease and neurodegeneration; inhibition or activation of follicle-stimulating hormone (modulation of fertility); chemotactic/chemokinetic activity (e.g. for mobilising specific cell types to sites of injury or infection); haemostatic and thrombolytic activity (e.g. for treating haemophilia and stroke); antiinflammatory activity (for treating e.g. septic shock or Crohn's disease); as antimicrobials; modulators of e.g. metabolism or behaviour; as analgesics; treating specific deficiency disorders; in treatment of e.g. psoriasis, in human or veterinary medicine.

In addition, or in the alternative, the composition of the present invention may be useful in the treatment of disorders listed in WO-A-98/09985. For ease of reference, part of that list is now provided: macrophage inhibitory and/or T cell inhibitory activity and thus, anti-inflammatory activity; anti-immune activity, i.e. inhibitory effects against a cellular and/or humoral immune response, including a response not associated with inflammation; inhibit the ability of macrophages and T cells to adhere to extracellular matrix components and fibronectin, as well as up-regulated fas receptor expression in T cells; inhibit unwanted immune reaction and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases, inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular inflammatory disorders, respiratory distress syndrome or other cardiopulmonary diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other glandular diseases, glomerulonephritis or other renal and urologic diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal diseases, periodontal diseases or other dental diseases, orchitis or epididimo-orchitis, infertility, orchidal trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic neuritis, intraocular inflammation, e.g. retinitis or cystoid macular oedema, sympathetic ophthalmia, scleritis, retinitis pigmentosa, immune and inflammatory components of degenerative fondus disease, inflammatory components of ocular trauma, ocular inflammation caused by infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g. following glaucoma filtration operation, immune

and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy, chronic neuropathy, Guillaim-Barre syndrome, Sydenham chora, myasthenia gravis, pseudo-tumour cerebri, Down's Syndrome, Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side effects of surgery, bone marrow transplantation or other transplantation complications and/or side effects, inflammatory and/or immune complications and side effects of gene therapy, e.g. due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases, e.g. leukaemia, by reducing the amount of monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

## SUMMARY

In summation, the present invention provides compounds for use as steroid dehydrogenase inhibitors, and pharmaceutical compositions for the same.

## EXAMPLES

The present invention will now be described only by way of example.

## MATERIALS AND METHODS

### Materials

**Enzymes** - Rat livers and rat kidneys were obtained from normal Wistar rats (Harlan Olac, Bicester, Oxon,UK). Both the kidneys and livers were homogenised on ice in PBS-sucrose buffer (1g/ 10 ml) using an Ultra-Turrax. After the livers and kidneys were homogenised the homogenate was centrifuged for five minutes at 4000 rpm. The supernatant obtained was removed and stored in glass vials at -20°C. The amount of protein per µl of rat liver and kidney cytosol was determined using the Bradford method [14].

**Apparatus**

- Incubator: mechanically shaken water bath, SW 20, Germany.
- Evaporator, Techne Driblock DB 3A, UK
- TLC aluminium sheets 20 x 20 cm silica gel 60 $F_{254}$, Merck, Germany.
- Scintillation vials: 20 ml polypropylene vials with caps, SARSTEDT, Germany.
- Scintillation counter: Beckman LS 6000 SC, Beckman Instruments Inc., USA.

**Solutions**

- Assay medium: PBS-sucrose buffer, Dulbecco's Phosphate Buffered Saline, 1 tablet/100 ml with 0,25 M sucrose, pH 7,4 BDH Laboratory supplies, UK.
- Scintillation fluid: Ecoscint A (National Diagnostics, USA).
- Radioactive compound solutions: [1,2,6,7-$^{3}$H]-cortisol (Sp. Ac. 84 Ci/mmol) NEN Germany, [4-$^{14}$C]-cortisol (Sp. Ac. 53 mCi/mmol) NEN Germany.
- $CrO_3$ and Acetic acid (Sigma Chemical Co., UK).
- Extraction fluid: Di-ethylether, Fischer Chemicals, UK.
- Bradford Reagent solution: Coomassie Brilliant Blue G-250, 100 mg in 95% ethanol with 100 ml of phosphoric acid (85% w/v) diluted to 1 litre.

**Compounds**

- Inhibitors: compounds were synthesised in accordance with the synthetic routes below .
- Cofactor: NADPH and NADP, Sigma Chemical Co., UK.

Methods

**Synthesis of radio labelled cortisone**

Labelled cortisone is commercially not available. Therefore labelled cortisol (F) ($^{3}$H-F and $^{14}$C-F) was oxidised at the C-11 position with $CrO_3$ in order to synthesize to the corresponding labelled cortisone ($^{3}$H-E and $^{14}$C-E).

For this reaction F was oxidised in a 0,25% $CrO_3$ (w/v) dissolved in a 50% acetic-acid/distilled water (v/v) solution. The labelled F was then added to 1 ml of the $CrO_3$ solution, vortex mixed and put in an incubator for 20 minutes at 37°C. The aqueous reaction mixture was extracted twice with 4 ml of di-ethylether, the di-ethylether was then evaporated and the residue transferred to a TLC-plate, which was developed in the following system, chloroform : methanol 9:1 (v/v). Unlabelled cortisone (E) was also run on the TLC-plate to locate the position of the labelled steroids. After locating the spot of the labelled steroids this area is cut out from the TLC-plate and eluted with 0,5 ml of methanol.

**The amount of protein per μL of rat liver and rat kidney**

The amount of protein in rat liver and rat kidney needed to be determined. The experiment was done according to the Bradford method [15]. The following method was used: first a BSA (protein) solution was prepared (1 mg/ml). Protein solutions containing 10 to 100 μg protein were pipetted into tubes and volumes adjusted with distilled water. Then 5 ml of protein reagent was added to the tubes and vortex mixed. The absorbance was measured at 595 nm after 15 minutes and before 1 hour in 3 ml cuvettes against a reagent blank. The weight of the protein was plotted against the corresponding absorbance resulting in a standard curve used to determine the protein concentration in rat liver and rat kidney cytosols.

**Assay validation - Enzyme concentration and time-dependency of 11 β-HSD activity**

Before carrying out 11 β-HSD assays to examine the conversion E to F and F to E and the influence that different inhibitors have on these conversions the amount of rat liver

homogenate and rat kidney homogenate and their incubation time need to be determined.

11 β-HSD type 1 is the enzyme responsible for the conversion E to F and this type of enzyme is present in rat liver. The substrate solution used in this assay contained 70,000 cpm/ml $^{3}$H-E in PBS-sucrose and 0.5 µM of unlabelled E and co-factor NADPH (9 mg/10 ml of substrate solution). 1 ml of the substrate solution and the different amounts of rat liver homogenate was added to all tubes.

The amount of rat liver homogenate needed for an assay was determined by incubating the substrate solution with 25, 50, 100 and 150 µl for 30, 60, 90 and 120 minutes at 37°C in a water bath with the tubes being mechanically shaken. After the incubation 50 µL of recovery solution was added, containing about 8,000 cpm/ 50 µL of $^{14}$C-F and 50 µg/50 µL of unlabelled F for visualising the spot on the TLC-plate, to correct for the losses made in the next two steps. F was then extracted from the aqueous phase with 4 ml of ether (2 x 30 sec cycle, vortex mix). The aqueous phase was then frozen using dry-ice and the organic layer was decanted and poured into smaller tubes and evaporated. 6 drops of ether were then added to the small tubes to re-dissolve the residue which was transferred to an aluminium thin layer chromatography plate (TLC-plate). The TLC-plate was developed in a TLC tank under saturated conditions. The solvent system used was chloroform : methanol 9:1 (v/v). The F spots on the TLC-plate were visualised under UV- light and cut out from the TLC-plate ($R_f$=0.45). The spots from the TLC- plate were then put into scintillation vials and 0.5 ml of methanol was added to all vials to elute the radioactivity from the TLC-plate for 5 minutes. 10 ml of Ecoscint was added to the scintillation vials and they were put into the scintillation counter to count amount of product formed.

The same procedure was used for the 11 β-HSD type 2 assay, the conversion F to E, to determine the amount of rat kidney to be used and the incubation time. Except this time the substrate solution contained $^{3}$H-F and unlabelled F and the recovery contained $^{14}$C-E and unlabelled E and cortisone has a $R_f$ value of 0.65 on the TLC-plate.

**Assay procedure - The 11 β-HSD inhibitors**

In these assays the influence of different inhibitors on the 11 β-HSD activity both in reductive (type 1) and oxidative (type 2) directions were assessed. In the reductive direction E is the substrate and F the product and visa versa in the case of oxidation. The method described here is for the oxidative direction.

The substrate solution contained about 50,000 cpm/ml $^{3}$H-F in PBS-sucrose and 0.5 µM F. 1ml of the substrate solution was added to each tube, the inhibitors were also added, at a 10 µM concentration, to each tube except to the "control" and "blank" tubes. 150 µL was added to all tubes except to the blanks, this was done to correct for the amount of $^{3}$H-F spontaneously formed. The tubes were incubated for 60 minutes in a mechanically shaken water bath at 37°C. The amount of kidney liver homogenate and incubation time used resulted from the enzyme- and time-dependency assay. After incubation 50 µL of recovery was added to correct for the losses made in the next steps, containing 5000 cpm/50 µL of $^{14}$C-E and 50µg/50 µL of unlabelled E (to visualise the spot on the TLC-plate). The aqueous mixture was then extracted with 4 ml of ether (2 x 30 sec cycle, vortex mix). After freezing the aqueous phase, the ether (upper) layer was decanted into smaller tubes and evaporated at 45°C until completely dry. The residue was then re-dissolved in 6 drops of ether and transferred to a TLC-plate. The TLC-plate was developed in chloroform : methanol (9:1 v/v) solvent system, the TLC-plate ran for about 90 minutes until the solvent front had moved about 18 cm. The position of the product E was visualised under UV-light and cut out from the TLC-plate and put into scintillation vials. Radioactivity was eluted over 5 minutes with 0.5 ml methanol. 0.5 ml of PBS-sucrose and 10 ml of Ecoscint were then added and vortex mixed before counting in the scintillation counter. Before counting the samples, two total activity vials were prepared. These contained 0.5 ml of the substrate solution, 50 µL of the recovery, 0.5 ml of methanol and 10 ml of Ecoscint. These two total activity vials were needed to determine the amount of $^{14}$C-E and $^{3}$H-F added in the beginning to make the calculations.

In case of the reductive direction, E to F ,the same method was used. Only the substrate solution containing $^{3}$H-E and unlabelled E and the recovery containing $^{14}$C-F and unlabelled F are different to the method used in the oxidative direction.

After testing all the inhibitors at 10 µM a dose-response experiment was done for the

most potent 11 β-HSD type 1 and type 2 inhibitors. To look at the percentage of inhibition four different concentrations, 1, 5, 10 and 20 μM, were used. The method for both the rat liver, type 1 the reductive, and rat kidney, type 2 the oxidative, stay the same throughout the entire experiment.

RESULTS

**The amount of protein per μL of rat liver and rat kidney**

An initial experiment was carried out to determine the amount of protein in rat liver cytosol and rat kidney cytosol, to be added to each tube. Graph 1 shows the standard curve from which the amount of protein used in both experiments was calculated. The amount of protein added to each tube in the rat liver experiment was 75.5 μg (per 25 μL). In the rat kidney experiment the amount of protein added to each tube was 135.6 μg (per 150μL).

**Enzyme concentration and time-dependency of 11 β-HSD activity**

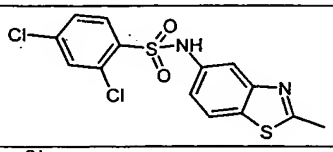
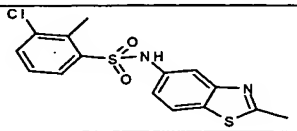
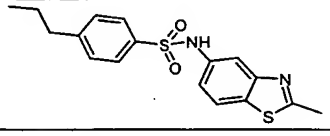
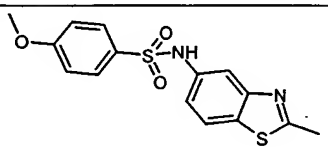
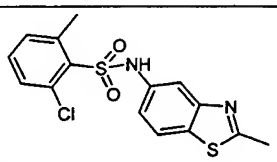
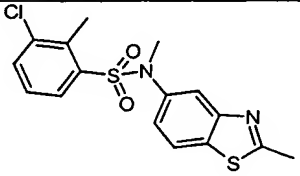
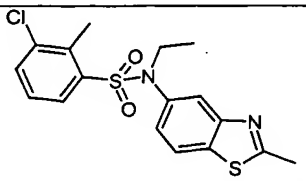
In this experiment the amount of rat liver homogenate and rat kidney homogenate added to each tube and the incubation time was determined. Graph 2 shows the enzyme concentration and time-dependency course of the rat liver experiment E to F, 11 β-HSD type 1 activity. Graph 3 shows the enzyme concentration and time-dependency course F to E, 11 β-HSD type 2 activity. After drawing the graphs the optimal amount of rat liver cytosol and rat kidney cytosol and both their incubation times were selected. One important rule when selecting both variables, to select an amount of rat liver and rat kidney and incubation time on a linear part of the graph. This is done to avoid fluctuations in enzyme activity. The amount of rat liver cytosol selected was 25 μL and 90 minutes of incubation time, the amount of rat kidney cytosol selected was 150 μL and 60 minutes of incubation time.

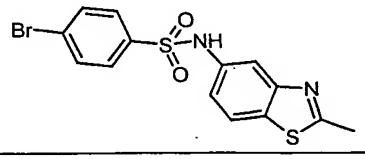
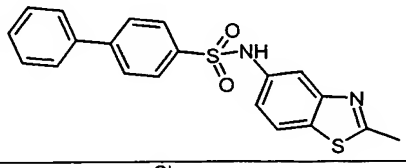
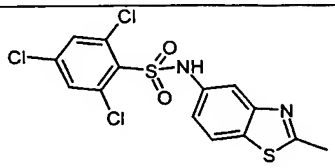
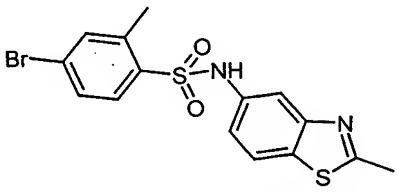
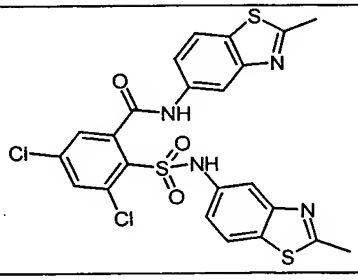
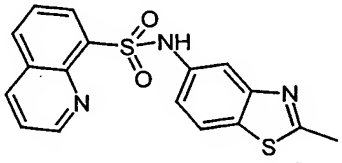
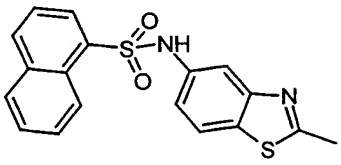
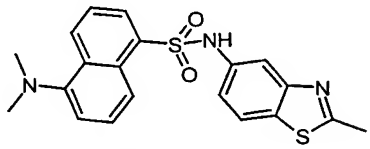
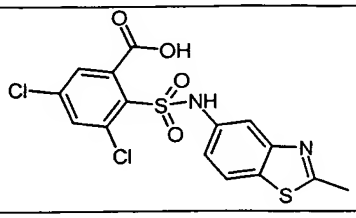
**The 11 β-HSD inhibitors**

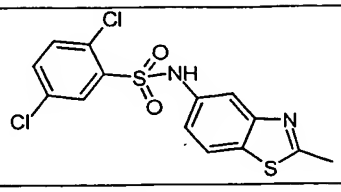
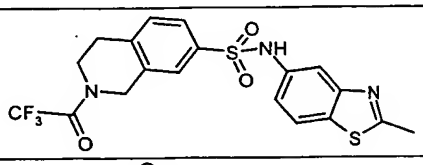
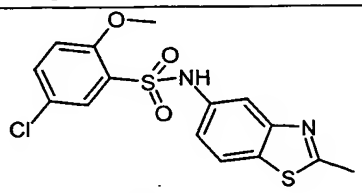
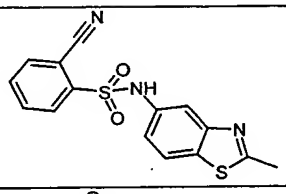
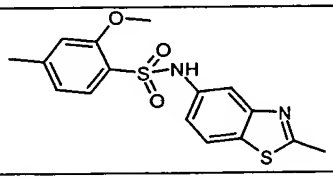
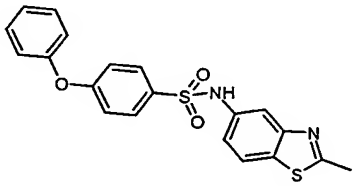
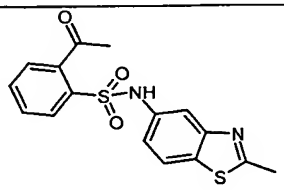
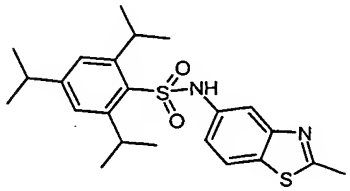
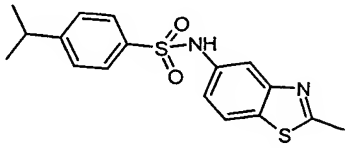
In this experiment the influence of different inhibitors on the conversion E to F and F to E was determined. The reason why inhibition in both directions was examined was to

make a comparison between the inhibitors and which type of 11 β-HSD they inhibit more. Compounds were screened for their ability to inhibit 11 β-HSD type 1 (E to F) and type 2 (F to E). All the inhibitors were initially tested at a 10 μM concentration. The percent of inhibition was calculated as the percentage of decrease in radio labelled $^{3}$H-E and $^{3}$H-F of product formed, compared with the control activity (the tubes without an inhibitor in it). All the results calculated are means, n=2.

**Table 1 - Inhibitory Effect**

| STX No. | Structure | % inhibition of 11β HSD1 @ 10 μM typical sd ± 5% | % inhibition of 11β HSD2 @ 10 μM typical sd ± 5% |
|---|---|---|---|
| 412 |  | 27 | 3 |
| 413 |  | 53 n=2<br>$IC_{50}$ = 6.6 μM | 0.2 |
| 421 |  | 60 n=2<br>$IC_{50}$ = 10 μM | 0.9 |
| 424 |  | 24 | 0.7 |
| 425 |  | 40 | 0.0 |
| 469 |  | 63 | 29 |
| 470 |  | 39 | 30 |

| | | | |
|---|---|---|---|
| 519 |  | 48 | 8 |
| 521 |  | 0.5 | 5 |
| 522 |  | 37 | 6 |
| 523 |  | 21 | 8 |
| 524 |  | 31 | 53 |
| 552 |  | 18 | 24 |
| 553 |  | 0.7 | 18 |
| 554 |  | 69 | 43 |
| 575 |  | 62 | 1.6 |

| | | | |
|---|---|---|---|
| 580 |  | 75 | 1.4 |
| 581 |  | 77 | 32 |
| 582 |  | 40 | 0.7 |
| 583 |  | 29 | 0.4 |
| 584 |  | 48 | 10 |
| 585 |  | 48 | 1.6 |
| 701 |  | 34 | 36 |
| 703 |  | 35 | 4 |
| 704 |  | 38 | 4 |

| | | | |
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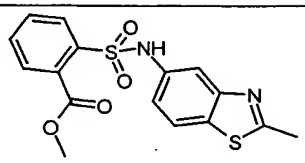
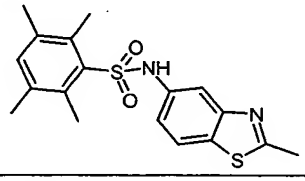
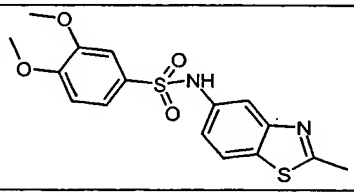
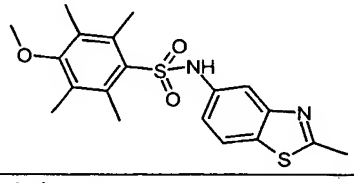
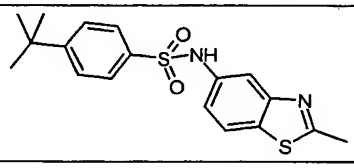
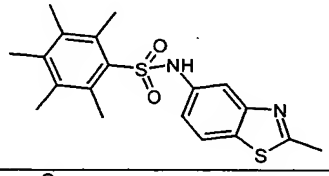
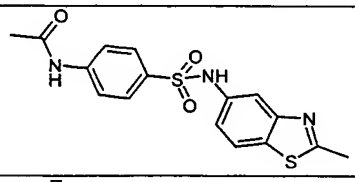
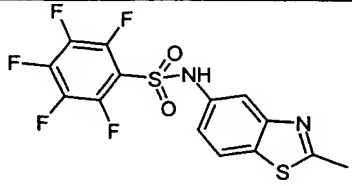
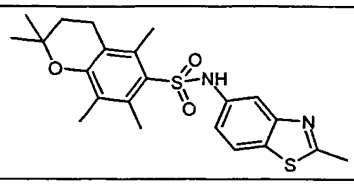
| 705 |  | 6 | 6 |
| 706 |  | 29 | 7 |
| 707 |  | 21 | 11 |
| 708 |  | 39 | 11 |
| 709 |  | 10 | 13 |
| 710 |  | 55 | 10 |
| 711 |  | 37 | 6 |
| 712 |  | 24 | 3 |
| 713 |  | 26 | 3 |

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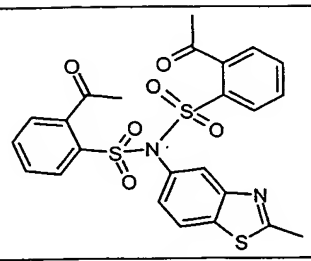
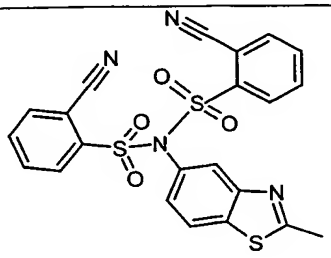
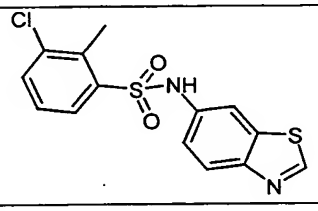
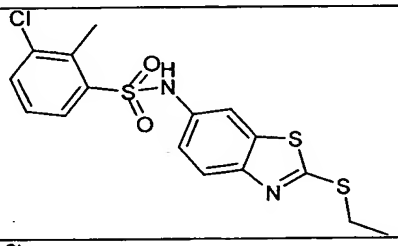
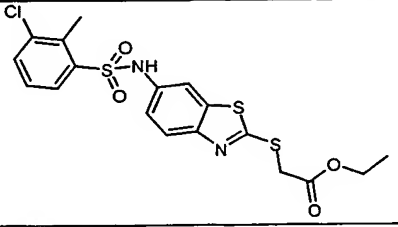
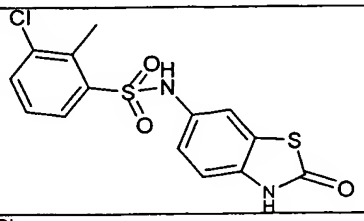
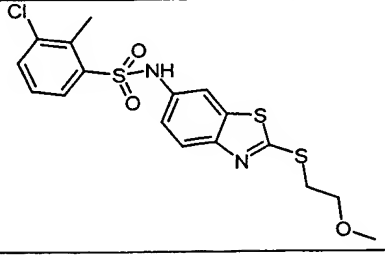
| 730 |  | 32 | 9 |
| 731 |  | 45 | 12 |
| 750 |  | 4 | 10 |
| 751 |  | 10 | 5 |
| 752 |  | 5 | 1 |
| 753 |  | 8 | 2 |
| 754 |  | 20 | 6 |

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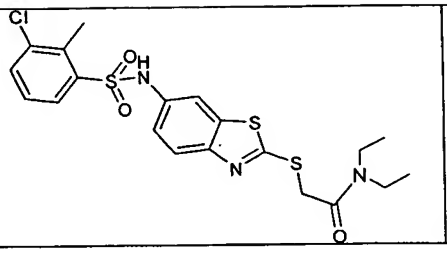
| 755 |  | 21 | 8 |

Sulphonamide synthesis

**Method A**

To the amine (1 eq.) dissolved in pyridine (3 eq.) was added the corresponding sulphonyl chloride (1.2 eq.) and the reaction mixture was stirred at RT under $N_2$ overnight. The resulting mixture was poured into aq. HCl and the organic layer was extracted with ethyl acetate, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the desired sulphonamide as crystalline solid or as a thick syrup. The crude compound was then purified by flash chromatography using EtOAc/hexane (3:2) or $CH_2Cl_2$/EtOAc (4:1) as eluent to give crystalline solid.

**Method B**

To the amine (1 eq.) dissolved in $Et_3N$ (5 eq.) was added the corresponding sulphonyl chloride (1.2 eq.) and the reaction mixture was stirred at RT under $N_2$ overnight. The resulting mixture was poured into water and the organic layer was extracted with ethyl acetate, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the desired sulphonamide as crystalline solid or as a thick syrup. The crude compound was then purified by flash chromatography using EtOAc/hexane (3:2) or $CH_2Cl_2$/EtOAc (4:1) as eluent to give crystalline solid.

**Note :** Insoluble amines and sulphonyl chlorides were dissolved in minimum amount of $CH_2Cl_2$, THF or DMF.

**Method C**

To a solution Arylsulphonyl chloride (1.1 eq.) in DCM were added Pyridine (2.2 eq.) and catalytic amount of DMAP. The solution was stirred at room temperature under nitrogen for 10 minutes. Then the amine (1 eq.) was added and the reaction mixture was stirred at

room temperature under nitrogen for 4~16 hrs. The resulting mixture was partitioned between DCM and 5% sodium bicarbonate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give a solid or a thick syrup. The crude compound was then purified by flash chromatography to give desired arylsulphonamide as crystalline solid.

**Library 2 (Novel Inhibitors) (34)**

**DGS03020A (STX412)**

Synthesised by method A. Off-white crystals of **DGS03020A** (186 mg; 55%). mp 189-190 $^0$C; TLC $R_f$ : 0.68 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 2.80 (s, 3H, $CH_3$), 7.13 (s, 1H, N-H, exchanged with $D_2O$), 7.212 (dd,1H, Ar-H, $J$ = 2.34 Hz and 8.59 Hz), 7.27 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 7.51 (d, 1H, Ar-H, $J$ = 1.95 *Hz*), 7.65 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.69 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.91 (d, 1H, Ar-H, $J$ = 8.59 Hz); MS (FAB+) 372.9 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 372.9627, $C_{14}H_{10}{}^{35}Cl_2N_2O_2S_2$ requires 372.9639, 376.9574, $C_{14}H_{10}{}^{37}Cl_2N_2O_2S_2$ requires 376.9580; HPLC $t_r$ 3.65 min (92 : 08 = MeOH : $H_2O$).

**DGS03022A (STX413)**

Synthesised by method A. Off-white crystals of **DGS03022A** (233 mg; 72%). mp 178 $^0$C; TLC $R_f$ : 0.71 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 2.75 (s, 3H, $CH_3$), 2.80 (s, 3H, $CH_3$), 6.75 (s, 1H, N-H, exchanged with $D_2O$), 7.11 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 7.17 – 7.21 (m, 1H, Ar-H), 7.53 (d, 1H, Ar-H, $J$ = 1.17 Hz), 7.55 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.68 (d, 1H, Ar-H, $J$ = 8.20 Hz), 7.92 (dd, 1H, Ar-H, $J$ = 1.17 Hz and 7.81 Hz); MS (FAB+) 164.1 [35, (5-Amino-2-methyl benzothiazole)$^+$], 353.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 353.0176, $C_{15}H_{14}{}^{35}ClN_2O_2S_2$ requires 353.0185, 355.0155, $C_{15}H_{14}{}^{37}ClN_2O_2S_2$ requires 355.0156; HPLC $t_r$ 3.78 min (92 : 08 = MeOH : $H_2O$).

**DGS03024A (STX421)**

Synthesised by method A. White crystals of **DGS03024A** (240 mg; 76%). mp 133-134 $^0$C; TLC $R_f$ : 0.7 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 0.90 (t, 3H, $C\underline{H_3}CH_2CH_2$, $J$ = 7.42 Hz), 1.56 – 1.66 (m, 2H, $CH_3\underline{CH_2}CH_2$), 2.59 (t, 2H, $CH_3CH_2\underline{CH_2}$, $J$ = 7.42 Hz), 2.80

(s, 3H, $CH_3$), 6.71 (s, 1H, N-H, exchanged with $D_2O$), 7.17 (d 1H, Ar-H, $J$ = 2.34 Hz and 8.59 Hz), 7.201 – 7.214 (m, 1H, Ar-H), 7.218- 7.223 (m, 1H, Ar-H), 7.57 (d, 1H, Ar-H, $J$ = 2.34 Hz), 7.76-7.69 (m, 3H, Ar-H); MS (FAB+) 347.1 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 347.0881, $C_{17}H_{19}N_2O_2S_2$ requires 347.0887; HPLC $t_r$ 3.69 min (92 : 08 = MeOH : $H_2O$).

**DGS03034A (STX424)**

Synthesised by method A. White crystals of **DGS03034A** (262 mg; 86%). mp 152 $^0$C; TLC $R_f$ : 0.48 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 10.31 (s, 1H, NH, Ex. With $D_2O$), 7.85 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.66 – 7.69 (m, 2H, Ar-H), 7.57 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.11 (dd, 1H, Ar-H, $J$ = 2.34 Hz and 8.59 Hz), 7.02 – 7.05 (m, 2H, Ar-H), 3.76 (s, 3H, $OCH_3$), 2.73 (s, 3H, $CH_3$); MS (FAB+) 164.0[25 (Amine $SM^+$)], 335.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 335.0519, $C_{15}H_{15}N_2O_3S_2$ requires 335.0524; HPLC $t_r$ 1.94 min (80 : 20 = MeOH : $H_2O$).

**DGS03036A (STX425)**

Synthesised by method A. White crystals of **DGS03036A** (136 mg; 42%). mp 295-296 $^0$C; TLC $R_f$ : 0.56 EtOAc/Hexane (3:2); $^1$H NMR (DMSO-$d_6$) 10.66 (s, 1H, NH, Ex. With $D_2O$), 7.87 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.52 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.32 – 7.44 (m, 3H, Ar-H), 7.12 (dd, 1H, Ar-H, $J$ = 2.3 Hz and 8.59 Hz), 2.73 (s, 3H, $CH_3$), 2.64 (s, 3H, $CH_3$); MS (FAB+) 164.0 [40, (Starting amine)$^+$], 353.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 353.0187, $C_{15}H_{14}{}^{35}ClN_2O_2S_2$ requires 353.0185, 355.0165, $C_{15}H_{14}{}^{37}ClN_2O_2S_2$ requires 355.0155; HPLC $t_r$ 1.94 min (80 : 20 = MeOH : $H_2O$).

**DGS03058A (STX519)**

Synthesised by method A. White crystals of **DGS03058A** (199 mg; 57%). mp 172 $^0$C; TLC $R_f$ : 0.56 EtOAc/Hexane (3:2); $^1$H NMR (DMSO-$d_6$) 10.53 (s, 1H, NH, Ex. With $D_2O$), 7.88 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.74 – 7.77 (m, 2H, Ar-H), 7.64 – 7.68 (m, 2H, Ar-H), 7.58 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.11 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 2.74 (s, 3H, $CH_3$); MS (FAB+) 384.9 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 384.9494,

$C_{14}H_{12}{}^{81}BrN_2O_2S_2$ requires 384.9503, 382.9501, $C_{14}H_{12}{}^{79}BrN_2O_2S_2$ requires 382.9523; HPLC $t_r$ 2.64 min (90 : 10 = MeOH : $H_2O$).

**DGS03062B (STX469)**

To a stirred solution of **DGS03022A** (50 mg, 0.14 mmol, 1 eq.) in anhy. DMF (5 ml) and NaH (7 mg, 0.16 mmol, 1.1 eq.) was added MeI (3 ml, 0.21 mmol, 1.5 eq.) and the mixture was stirred for 1h. The resulting mixture was poured into water and the organic layer was extracted with ethyl acetate, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a yellow suspension. The crude compound (70 mg) was purified by flash chromatography using EtOAc/hexane (3:2) as eluent to give white crystals of **DGS03062A** (36 mg; 69%). mp 97-98 $^0$C; TLC $R_f$ : 0.61 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 7.73 (dd, 1H, Ar-H, $J$ = 1.17 Hz and 7.81 Hz), 7.37 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.59 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.49 (dd, 1H, Ar-H, $J$ = 1.17 Hz and 8.2 Hz), 7.24 (dd, 1H, Ar-H, $J$ = 2.34 Hz and 8.59 Hz), 7.11 – 7.15 (m, 1H, Ar-H), 3.24 (s, 3H, $CH_3$), 2.76 (s, 3H, $CH_3$), 2.35 (s, 3H, $CH_3$); MS (FAB+) 366.9 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 366.0262, $C_{16}H_{15}{}^{35}ClN_2O_2S_2$ requires 366.0262, 368.0300, $C_{16}H_{15}{}^{37}ClN_2O_2S_2$ requires 368.0234; HPLC $t_r$ 1.93 min (96 : 04 = MeOH : $H_2O$).

**DGS03072A (STX470)**

To a stirred solution of **DGS03022A** (50 mg, 0.14 mmol, 1 eq.) in anhy. DMF (5 ml) and NaH (10 mg, 0.16 mmol, 1.1 eq.) was added EtI (23 mg, 0.21 mmol, 1.5 eq.) and the mixture was stirred for 1h. The resulting mixture was poured into water and the organic layer was extracted with ethyl acetate, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a yellow suspension. The crude compound (75 mg) was purified by flash chromatography using EtOAc/hexane (3:2) as eluent to give a pale yellow thick syrup of **DGS03072A** (16 mg; 30%). TLC $R_f$ : 0.71 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 7.76 – 7.78 (m, 2H, Ar-H), 7.66 (m, 1H, Ar-H), 7.53 – 7.55 (m, 1H, Ar-H), 7.27 – 7.28 (m, 1H, Ar-H), 7.14 – 7.18 (m, 1H, Ar-H), 7.11 – 7.18 (m, 1H, Ar-H), 5.30 (s, 1H, NH, Ex. with $D_2O$), 3.74 (q, 2H, Ar-H, $J$ = 7.42 Hz and 7.03 Hz), 2.83 (s, 3H, $CH_3$), 2.53 (s, 3H, $CH_3$), 1.12 (t, 3H, $CH_3$, $J$ = 7.03 Hz), MS (FAB+) 381.1 [100, $(M+H)^+$];

HRMS *m/z* (FAB+) 381.1062, $C_{17}H_{17}{}^{35}ClN_2O_2S_2$ requires 381.1058, 385.0952, $C_{17}H_{17}{}^{37}ClN_2O_2S_2$ requires 385.0949.

**DGS03082A (STX521)**

Synthesised by method A. White crystals of **DGS03082A** (230 mg; 67%). mp 85-86 $^0$C; TLC $R_f$ : 0.64 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 10.54 (s, 1H, NH, Ex. With $D_2O$), 7.87 (d, 1H, Ar-H, *J* = 8.59 Hz), 7.84 (broad s, 4H, Ar-H), 7.67 – 7.69 (m, 2H, Ar-H), 7.62 (d, 1H, Ar-H, *J* = 1.95 Hz), 7.39 – 7.49 (m, 3H, Ar-H), 7.17 (dd, 1H, Ar-H, *J* = 1.95 Hz and 8.59 Hz), 2.73 (s, 3H, $CH_3$); MS (FAB+) 381.2 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 381.0730, $C_{20}H_{17}N_2O_2S_2$ requires 381.0731; HPLC $t_r$ 1.36 min (96 : 04 = MeOH : $H_2O$).

**DGS03084A (STX522)**

Synthesised by method A. Yellow crystals of **DGS03084A** (46 mg; 10%). mp 253-254 $^0$C; TLC $R_f$ : 0.74 EtOAc/Hexane (3:2); $^1$H NMR (DMSO-$d_6$) 11.09 (s, 1H, NH, Ex. with $D_2O$), 7.91 (d, 1H, Ar-H, *J* = 8.59 Hz), 7.86 (s, 2H, Ar-H), 7.59 (d, 1H, Ar-H, *J* = 2.34 Hz), 7.15 (dd, 1H, Ar-H, *J* = 1.95 Hz and 8.59 Hz), 2.74 (s, 3H, $CH_3$); MS (FAB+) 409.1 [100, $(M+H)^+$]; MS (FAB-) 407.0 [100, $(M-H)^+$]; HRMS *m/z* (FAB+) 406.9176, $C_{14}H_{19}{}^{35}Cl_3N_2O_2S_2$ requires 406.9167, 408.9136, $C_{14}H_{19}{}^{37}Cl_3N_2O_2S_2$ requires 408.9140.

**DGS03086A (STX523)**

Synthesised by method A. Pale yellow crystals of **DGS03086A** (101 mg; 57%). mp 219 $^0$C; TLC $R_f$ : 0.71 EtOAc/Hexane (3:2); $^1$H NMR (DMSO-$d_6$) 10.68 (s, 1H, NH, Ex. With $D_2O$), 7.87 (d, 1H, Ar-H, *J* = 8.59 Hz), 7.79 (d, 1H, Ar-H, *J* = 8.59 Hz), 7.64 (d, 1H, Ar-H, *J* = 1.95 Hz), 7.54 – 7.57 (m, 2H, Ar-H), 7.11 (dd, 1H, Ar-H, *J* = 2.34 Hz and 8.59 Hz), 2.73 (s, 3H, $CH_3$), 2.59 (s, 3H, $CH_3$); MS (FAB+) 399.0 [100, $(M+H)^+$], 164.1 [50, (Starting amine)$^+$]; HRMS *m/z* (FAB+) 398.9663, $C_{15}H_{13}{}^{81}BrN_2O_2S_2$ requires 398.9569, 396.9684, $C_{15}H_{13}{}^{79}BrN_2O_2S_2$ requires 396.9680; HPLC $t_r$ 1.39 min (96 : 04 = MeOH : $H_2O$).

**DGS03064**

2,4-Dichloro benzoic acid (10 g, 0.0523 mol, 1 eq.) was heated to 115 $^0$C with excess chlorosulphonic acid (10.5 mL, 0.1571 mol, 3 eq.) under $N_2$ for 18 h. The resulting mixture was cooled and consciously poured into ice-water. The resulted white precipitate was filtered out, washed with plenty of water and dried under vacuum over night. The crude **DGS03064** (11.5 g, 76 %) was used for the subsequent reaction without further purification. mp 173-174 $^0$C; TLC $R_f$ ; 0.48 (4:1, $CH_2Cl_2$/EtOAc); $^1$H NMR ($CDCl_3$) $\delta$ 8.28 (1H, s, Ar-H), 7.65 (1H, s, Ar-H); MS *m/z* (FAB+) 286.9 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 287.8798, $C_7H_3{}^{35}Cl_3O_4S$ requires 287.8818, 291.8755, $C_7H_3{}^{37}Cl_3O_4S$ requires 291.8759.

**DGS03088A (STX524)**

Synthesised by method B. Two compounds were isolated - DGS03088A and DGS03088A. White crystals of **DGS03088A** (48 mg; 13%). mp 153-155 $^0$C; TLC $R_f$ : 0.79 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 8.31 (s, 1H, NH, Ex. With $D_2O$), 8.07 (s, 1H, NH, Ex. With $D_2O$), 8.07 (s, 1H, Ar-H), 7.71 – 7.79 (m, 4H, Ar-H), 7.67 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.58 (s, 1H, Ar-H), 7.27 (dd, 1H, Ar-H, $J$ = 2.72 Hz and 8.59 Hz), 2.83 (s, 3H, $CH_3$), 2.79 (s, 3H, $CH_3$); MS (FAB+) 562.9 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 562.9825, $C_{23}H_{17}{}^{35}Cl_2N_4O_3S_3$ requires 562.9839, 566.9778, $C_{23}H_{17}{}^{37}Cl_2N_4O_3S_3$ requires 566.9781; HPLC $t_r$ 1.33 min (96 : 04 = MeOH : $H_2O$).

**DGS03088-1 (STX575)**

White crystals of **DGS03088-1** (31 mg; 12%). mp 147-148 $^0$C; TLC $R_f$ : 0.45 EtOAc/Hexane (3:2); $^1$H NMR ($CDCl_3$) 8.45 (s, 1H, NH, Ex. With $D_2O$), 8.17 (d, 1H, Ar-H, $J$ = 8.09 Hz), 8.04 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.50 (d, 1H, Ar-H, $J$ = 1.83 Hz), 7.35 (dd, 1H, Ar-H, $J$ = 1.83 Hz and 8.05 Hz), 2.85 (s, 3H, $CH_3$); LC-MS 418.1 [100, $(M^+)$]; HRMS *m/z* (FAB+) -----, $C_{15}H_{11}{}^{35}Cl_2N_2O_4S_2$ requires ---, ----, $C_{15}H_{11}{}^{37}Cl_2N_2O_4S_2$ requires ---; HPLC $t_r$ 1.97 min (96 : 04 = MeOH : $H_2O$).

**DGS03100A (STX552)**

Synthesised by method B. White crystals of **DGS03100A** (224 mg; 69%). mp 222-223 $^0$C; TLC $R_f$ : 0.56 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.27 (s, 1H, NH, Ex. With $D_2O$), 9.16 – 9.17 (m, 1H, Ar-H), 8.48 – 8.51 (m, 2H, Ar-H), 8.36 – 8.38 (m, 2H, Ar-H), 8.23 – 8.25 (m, 1H, Ar-H), 7.67 – 7.34 (m, 3H, Ar-H), 7.51 – 7.12 (m, 1H, Ar-H), 7.09 – 7.12 (m, 1H, Ar-H), 2.67 (s, 3H, $CH_3$); LC-MS 355.7 [$(M)^+$]; MS (FAB+) 356.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 356.0531, $C_{17}H_{14}N_3O_2S_2$ requires 356.0527; HPLC $t_r$ 1.86 min (96 : 04 = MeOH : $H_2O$).

**DGS03102A (STX553)**

Synthesised by method B. Pale yellow crystals of **DGS03102A** (170 mg; 52%). mp 89-90 $^0$C; TLC $R_f$ : 0.55 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.87 (s, 1H, NH, Ex. With $D_2O$), 8.28 – 8.24 (m, 1H, Ar-H), 8.06 – 8.22 (m, 2H, Ar-H), 8.05 (d, 1H, Ar-H, $J$ = 8.20 Hz), 7.60 – 7.77 (m, 2H, Ar-H), 7.47 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.04 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 2.69 (s, 3H, $CH_3$); MS (FAB+) 355.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 355.0576, $C_{18}H_{15}N_2O_2S_2$ requires 355.0575; HPLC $t_r$ 1.93 min (96 : 04 = MeOH : $H_2O$).

**DGS03104A (STX554)**

Synthesised by method B. Yellow crystals of **DGS03104A** (230 mg; 63%). mp 85-86 $^0$C; TLC $R_f$ : 0.65 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.84 (s, 1H, NH, Ex. With $D_2O$), 8.40 – 8.42 (m, 2H, Ar-H), 8.22 – 8.23 (m, 1H, Ar-H), 7.76 – 7.78 (m, 1H, Ar-H), 7.58 – 7.65 (m, 2H, Ar-H), 7.51 – 7.56 (m, 1H, Ar-H), 7.23 – 7.25 (m, 1H, Ar-H), 7.05 – 7.07 (m, 1H, Ar-H), 2.79 (s, 6H, 2×$CH_3$), 2.69 (s, 3H, $CH_3$); MS (FAB+) 398.1 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 398.0978, $C_{20}H_{20}N_3O_2S_2$ requires 398.0997; HPLC $t_r$ 2.01 min (96 : 04 = MeOH : $H_2O$).

**DGS03116A (STX580)**

Synthesised by method B. Pale yellow crystals of **DGS03116A** (151 mg; 44%). mp 153 $^0$C; TLC $R_f$ : 0.55 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.96 (s, 1H, NH, Ex. With $D_2O$), 8.00 (d, 1H, Ar-H, $J$ = 2.34 Hz), 7.90 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.66 – 7.73 (m,

2H, Ar-H), 7.58 (d, 1H, Ar-H, $J$ = 2.34 Hz), 7.17 (dd, 1H, Ar-H, $J$ = 2.34 Hz and 8.59 Hz), 2.74 (s, 3H, $CH_3$); MS (FAB+) 372.8 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 375.9599, $C_{14}H_{11}{}^{37}Cl_2N_2O_2S_2$ requires 375.9502, 372.9606, $C_{14}H_{11}{}^{35}Cl_2N_2O_2S_2$ requires 372.9639; HPLC $t_r$ 2.98 min (90 : 10 = MeOH : $H_2O$).

**DGS03118A (STX581)**

Synthesised by method B. White crystals of **DGS03118A** (416 mg; 42%). mp 88-89 $^0$C; TLC $R_f$ : 0.49 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.47 (s, 1H, NH, Ex. With $D_2O$), 7.74 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.58 – 7.61 (m, 2H, Ar-H), 7.35 – 7.38 (m, 1H, Ar-H), 7.13 – 7.17 (m, 1H, Ar-H), 4.76 – 4.78 (m, 2H, $CH_2$), 3.75 – 3.79 (m, 2H, $CH_2$), 2.90 – 2.93 (m, 2H, $CH_2$), 2.73 (s, 3H, $CH_3$); MS (FAB+) 456.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 456.0663, $C_{19}H_{17}F_3N_3O_3S_2$ requires 456.0663; HPLC $t_r$ 1.63 min (96 : 04 = MeOH : $H_2O$).

**DGS03120A (STX582)**

Synthesised by method B. Pale yellow crystals of **DGS03120A** (185 mg; 55%). mp 91-92 $^0$C; TLC $R_f$ : 0.51 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.35 (s, 1H, NH, Ex. With $D_2O$), 7.85 (d, 1H, Ar-H, $J$ = 8.98 Hz), 7.69 (d, 1H, Ar-H, $J$ = 2.34 Hz), 7.61 (dd, 1H, Ar-H, $J$ = 2.73 Hz and 8.98 Hz), 7.55 (d, 1H, Ar-H, $J$ = 2.73 Hz), 7.20 (d, 1H, Ar-H, $J$ = 8.98 Hz), 7.15 (dd, 1H, Ar-H, $J$ = 2.3 Hz and 8.59 Hz), 3.89 (s, 3H, $OCH_3$), 2.73 (s, 3H, $CH_3$); MS (FAB+) 369.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 371.0114, $C_{15}H_{14}{}^{37}ClN_2O_3S_2$ requires 371.0105, 369.0135, $C_{15}H_{14}{}^{35}ClN_2O_3S_2$ requires 369.0134; HPLC $t_r$ 1.68 min (96 : 04 = MeOH : $H_2O$).

**DGS03122A (STX731)**

Synthesised by method B. Two compounds were isolated - DGS03122A and DGS03122B. Yellow crystals of **DGS03122A** (67 mg; 22%). mp 272-273 $^0$C; TLC $R_f$ : 0.59 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 8.15 (m, 5H, , Ar-H), 8.02 – 8.09 (m, 4H, Ar-H), 7.74 (d, 1H, Ar-H, $J$ = 2.3 Hz), 7.14 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 2.84 (s, 3H, $CH_3$); MS (FAB+) 495.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) -----, $C_{22}H_{14}N_4O_4S_3$ requires -----; HPLC $t_r$ 1.79 min (90 : 10 = MeOH : $H_2O$).

**DGS03122B (STX583)**

Yellow crystals of **DGS03122B** (47 mg; 16%). mp 204-206 $^0$C; TLC $R_f$ : 0.48 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.95 (s, 1H, NH, Ex. With $D_2O$), 8.03 – 8.07 (m, 2H, Ar-H), 7.86 – 7.91 (m, 2H, Ar-H), 7.77 – 7.81 (m, 1H, Ar-H), 7.55 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.12 (dd, 1H, Ar-H, $J$ = 2.34 Hz and 8.59 Hz), 2.74 (s, 3H, $CH_3$); MS (FAB+) 330.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 330.0370, $C_{15}H_{12}N_3O_2S_2$ requires 330.0371; HPLC $t_r$ 1.84 min (90 : 10 = MeOH : $H_2O$).

**DGS03124A (STX584)**

Synthesised by method B. Pale yellow crystals of **DGS03124A** (125 mg; 55%). mp 188-189 $^0$C; TLC $R_f$ : 0.37 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.09 (s, 1H, NH, Ex. With $D_2O$), 7.81 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.63 (d, 1H, Ar-H, $J$ = 8.20 Hz), 7.56 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.14 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 6.96 (s, 1H, Ar-H), 6.81 (d, 1H, Ar-H, $J$ = 8.59 Hz), 3.87 (s, 3H, $OCH_3$), 2.72 (s, 3H, $CH_3$), 2.28 (s, 3H, $CH_3$); MS (FAB+) 219.1 [20, (sulphonyl chloride-H)$^+$], 349.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 349.0678, $C_{16}H_{17}N_2O_3S_2$ requires 349.0681; HPLC $t_r$ 1.80 min (96 : 04 = MeOH : $H_2O$).

**DGS03126A (STX585)**

Synthesised by method B. Pale yellow crystals of **DGS03126A** (145 mg; 40%). mp 84-86 $^0$C; TLC $R_f$ : 0.71 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.42 (s, 1H, NH, Ex. With $D_2O$), 7.88 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.73 – 7.77 (m, 2H, Ar-H), 7.59 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.41 – 7.46 (m, 2H, Ar-H), 7.22 – 7.26 (m, 2H, Ar-H), 7.13 (dd, 1H, Ar-H, $J$ = 8.59 Hz and 2.34 Hz), 7.02 – 7.10 (m, 4H, Ar-H), 2.75 (s, 3H, $CH_3$); MS (FAB+) 397.0 [100, $(M+H)^+$]; HRMS *m/z* (FAB+) 397.0671, $C_{20}H_{17}N_2O_3S_2$ requires 397.0681; HPLC $t_r$ 1.93 min (96 : 04 = MeOH : $H_2O$).

**DGS03130A (STX730)**

Synthesised by method B. Two compounds were isolated - DGS03130A and DGS03130B. Synthesised by method B. Pale yellow crystals of **DGS03130A** (105 mg;

33%). mp 125-126 $^0$C; TLC $R_f$ : 0.55 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 8.21 – 8.24 (m, 4H, Ar-H), 8.13 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.99 – 8.03 (m, 4H, Ar-H), 7.57 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.03 (dd, 1H, Ar-H, $J$ = 8.59 Hz and 1.95 Hz), 2.82 (s, 3H, $CH_3$); 2.69 (s, 6H, 2×$CH_3$); MS (FAB+) 529.0 [100, $(M+H)^+$]; MS (FAB-) 527.1 [70, $(M-H)^+$], 345.0 [100, (M-2-Acetyl sulphonyl chloride)$^+$]; HRMS *m/z* (FAB+) ----, $C_{24}H_{20}N_2O_6S_3$ requires ---; HPLC $t_r$ 1.81 min (96 : 04 = MeOH : $H_2O$).

**DGS03130B (STX701)**

Pale yellow crystals of **DGS03130A** (45 mg; 14%). mp 169 $^0$C; TLC $R_f$ : 0.42 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.63 (s, 1H, NH, Ex. With $D_2O$), 8.04 – 8.07 (m, 2H, Ar-H), 7.86 – 7.89 (m, 3H, Ar-H), 7.59 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.13 (dd, 1H, Ar-H, $J$ = 8.9 Hz and 2.3 Hz), 3.73 (s, 3H, $CH_3$); 2.56 (s, 3H, $CH_3$); MS (FAB+) 347.0 [100, $(M+H)^+$], 219.1 [10, (sulphonyl chloride+H)$^+$]; HRMS *m/z* (FAB+) 347.0522, $C_{16}H_{15}N_2O_3S_2$ requires 347.0524; HPLC $t_r$ 1.77 min (96 : 04 = MeOH : $H_2O$).

**DGS03134A (STX703)**

Synthesised by method B. Pale yellow crystals of **DGS03134A** (91 mg; 23%). mp 206-207 $^0$C; TLC $R_f$ : 0.81 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR (DMSO-$d_6$) 10.37 (s, 1H, NH, Ex. With $D_2O$), 7.87 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.46 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.19 (s, 2H, Ar-H), 7.07 (dd, 1H, Ar-H, $J$ = 8.59 Hz and 1.95 Hz), 4.13 – 4.20 (m, 2H, 2×$(CH_3)_2\underline{H}$), 2.83 – 2.89 (m, 1H, $(CH_3)_2\underline{H}$), 2.72 (s, 3H, $CH_3$), 1.15 (d, 12H, 4×$(CH_3)_2$, $J$ = 7.03 Hz), 1.11 (d, 9H, 2×$(CH_3)_2$, $J$ = 6.64 Hz); LC-MS 429.72 $(M)^+$; HRMS *m/z* (FAB+) ---, $C_{23}H_{31}N_2O_4S_2$ requires ----; HPLC $t_r$ 2.84 min (90 : 10 = MeOH : $H_2O$).

**DGS03136A (STX704)**

Synthesised by method B. Pale yellow crystals of **DGS03136A** (225 mg; 71%). mp 54-55 $^0$C; TLC $R_f$ : 0.50 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.65 (m, 3H, Ar-H), 7.58 (d, 1H, Ar-H, $J$ = 2.34 Hz), 7.18 (dd, 1H, Ar-H, $J$ = 8.6 Hz and 1.95 Hz), 6.84 – 6.85 (m, 2H, Ar-H), 6.82 (s, 1H, NH, Ex. With $D_2O$), 4.51 – 4.60 (m, 1H, $(CH_3)_2\underline{H}$), 2.80 (s, 3H, $CH_3$), 1.31 (s, 6H, $(CH_3)_2$); LC-MS 347.6 $(M)^+$; HRMS *m/z* (FAB+) 347.0847, $C_{17}H_{19}N_2O_2S_2$ requires 347.0837; HPLC $t_r$ 2.39 min (90 : 10 = MeOH : $H_2O$).

**DGS03138B (STX705)**

Synthesised by method B. Pale yellow crystals of **DGS03138B** (24 mg; 7%). mp 248 $^0$C; TLC $R_f$ : 0.52 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 8.18 (d, 1H, Ar-H, $J$ = 8.59 Hz), 8.15 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.89 – 8.04 (m, 4H, Ar-H), 7.51 (dd, 1H, Ar-H, $J$ = 8.20 Hz and 1.95 Hz), 7.27 (s, 1H, NH, Ex. With $D_2O$), 2.89 (s, 3H, $CH_3$), 1.59 (s, 3H, $CH_3$); LC-MS 372.90 $(M+CH_3CN)^+$; HRMS *m/z* (FAB+) 371.2281, $C_{16}H_{15}N_2O_4S_2$ requires 371.2278; HPLC $t_r$ 2.22 min (90 : 10 = MeOH : $H_2O$).

**DGS03140A (STX711)**

Synthesised by method B. Brown crystals of **DGS03140A** (85 mg; 26%). mp 73-75 $^0$C; TLC $R_f$ : 0.59 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.85 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.80 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.68 (s, 1H, NH, Ex. With $D_2O$), 7.57 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.54 (s, 1H, NH, Ex. With $D_2O$), 7.24 (d, 1H, Ar-H, $J$ = 2.34 Hz), 7.18 (dd, 1H, Ar-H, $J$ = 8.20 Hz and 1.95 Hz), 7.03 (dd, 1H, Ar-H, $J$ = 8.59 Hz and 2.34 Hz), 6.77 (dd, 1H, Ar-H, $J$ = 8.59 Hz and 2.34 Hz), 2.78 (s, 3H, $CH_3$), 2.24 (s, 3H, $CH_3$); LC-MS 362.32 $(M)^+$; HRMS *m/z* (FAB+) 361.0587, $C_{16}H_{16}N_3O_3S_2$ requires 361.0636; HPLC $t_r$ 2.09 min (90 : 10 = MeOH : $H_2O$).

**DGS03142A (STX706)**

Synthesised by method B. Pale yellow crystals of **DGS03142A** (79 mg; 24%). mp 89-91 $^0$C; TLC $R_f$ : 0.65 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.78 (d, 1H, Ar-H, $J$ = 8.20 Hz), 7.61 (d, 1H, Ar-H, $J$ = 1.56 Hz), 6.98 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.20 Hz), 6.93 (s, 1H, Ar-H), 6.92 (s, 1H, NH, Ex. With $D_2O$), 3.99 (s, 6H, 2×$CH_3$), 3.93 (s, 6H, 2×$CH_3$), 2.85 (s, 3H, $CH_3$); LC-MS 361.48 $(M)^+$; HRMS *m/z* (FAB+) 361.1605, $C_{18}H_{21}N_2O_2S_2$ requires 361.1606; HPLC $t_r$ 2.26 min (90 : 10 = MeOH : $H_2O$).

**DGS03144A (STX707)**

Synthesised by method B. Pale yellow crystals of **DGS03144A** (79 mg; 24%). mp 89-91 $^0$C; TLC $R_f$ : 0.69 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.70 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.58 (d, 1H, Ar-H, $J$ = 2.34 Hz), 7.39 (dd, 1H, Ar-H, $J$ = 2.34 Hz and 8.59 Hz), 7.19

(d, 1H, Ar-H, $J$ = 1.95 Hz), 7.17 (t, 1H, Ar-H, $J$ = 1.95 Hz), 6.83 (d, 1H, Ar-H, $J$ = 8.59 Hz), 6.59 (s, 1H, NH, Ex. With $D_2O$), 3.89 (s, 3H, $OCH_3$), 3.76 (s, 3H, $OCH_3$), 2.81 (s, 3H, $CH_3$); LC-MS 363.02 $(M)^+$; HRMS *m/z* (FAB+) 365.0642, $C_{16}H_{17}N_2O_4S_2$ requires 365.0585; HPLC $t_r$ 2.15 min (90 : 10 = MeOH : $H_2O$).

**DGS03146A (STX708)**

Synthesised by method B. Pale yellow crystals of **DGS03146A** (181 mg; 51%). mp 175 $^0$C; TLC $R_f$ : 0.57 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.71 (dd, 1H, Ar-H, $J$ = 2.3 Hz and 8.98 Hz), 7.59 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.43 (d, 1H, Ar-H, $J$ = 8.98 Hz), 7.21 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 6.67 (s, 1H, NH, Ex. With $D_2O$), 2.81 (s, 3H, $OCH_3$), 1.59 (s, 6H, 2×$CH_3$), 1.29 (s, 6H, 2×$CH_3$); LC-MS 377.01 $(M)^+$; HRMS *m/z* (FAB+) 377.0988, $C_{18}H_{21}N_2O_3S_2$ requires 377.0994; HPLC $t_r$ 2.53 min (90 : 10 = MeOH : $H_2O$).

**DGS03148A (STX709)**

Synthesised by method B. Off-white crystals of **DGS03148A** (102 mg; 31%). mp 214-215 $^0$C; TLC $R_f$ : 0.62 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.71 – 7.73 (m, 2H, Ar-H), 7.60 – 7.61 (m, 1H, Ar-H), 7.44 – 7.46 (m, 2H, Ar-H), 7.21 – 7.24 (m, 2H, Ar-H), 6.61 (s, 1H, NH, Ex. With $D_2O$), 2.83 (s, 3H, $CH_3$), 1.31 (s, 9H, $(CH_3)_3$); LC-MS 360.12 $(M)^+$; HRMS *m/z* (FAB+) 361.1057, $C_{18}H_{21}N_2O_3S_2$ requires 361.1044; HPLC $t_r$ 2.67 min (90 : 10 = MeOH : $H_2O$).

**DGS03150A (STX710)**

Synthesised by method B. Pale yellow crystals of **DGS03150A** (101 mg; 30%). mp 200-201 $^0$C; TLC $R_f$ : 0.50 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.65 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.44 (d, 1H, Ar-H, $J$ = 1.95 Hz), 7.09 (dd, 1H, Ar-H, $J$ = 1.95 Hz and 8.59 Hz), 6.75 (s, 1H, NH, Ex. With $D_2O$), 2.79 (s, 3H, $CH_3$), 2.57 (s, 6H, 2×$CH_3$), 2.24 (s, 3H, $CH_3$), 2.19 (s, 6H, 2×$CH_3$); LC-MS 374.10 $(M)^+$; HRMS *m/z* (FAB+) 375.1195, $C_{19}H_{23}N_2O_2S_2$ requires 375.1201; HPLC $t_r$ 3.15 min (80 : 20 = MeOH : $H_2O$).

**DGS03152A (STX712)**

Synthesised by method B. Pale yellow crystals of **DGS03152A** (120 mg; 33%). mp 181-182 $^0$C; TLC $R_f$ : 0.65 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.63 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.59 (d, 1H, Ar-H, $J$ = 2.3 Hz), 7.22 (dd, 1H, Ar-H, $J$ = 2.3 Hz and 8.59 Hz), 4.91 (s, 1H, NH, Ex. With $D_2O$), 3.82 (s, 3H, $CH_3$); LC-MS 392.96 $(M)^+$; HRMS *m/z* (FAB+) 394.9941, $C_{14}H_8F_5N_2O_2S_2$ requires 394.9947; HPLC $t_r$ 2.49 min (90 : 10 = MeOH : $H_2O$).

**DGS03158A (STX713)**

Synthesised by method B. Yellow crystals of **DGS03158A** (158 mg; 40%). mp 334-335 $^0$C; TLC $R_f$ : 0.47 $CH_2Cl_2$/EtOAc (4:1); $^1$H NMR ($CDCl_3$) 7.65 (d, 1H, Ar-H, $J$ = 8.59 Hz), 7.47 (d, 1H, Ar-H, $J$ = 2.3 Hz), 7.10 (dd, 1H, Ar-H, $J$ = 2.3 Hz and 8.59 Hz), 6.69 (s, 1H, NH, Ex. With $D_2O$), 2.79 (s, 3H, $CH_3$), 2.61 (t, 2H, $CH_2$, $J$ = 6.64 Hz), 2.55 (s, 3H, $CH_3$), 2.51 (s, 3H, $CH_3$), 2.08 (s, 3H, $CH_3$), 1.79 (t, 2H, $CH_2$, $J$ = 7.03 Hz), 1.29 (s, 6H, 2×$CH_3$); LC-MS 431.11 $(M)^+$; HRMS *m/z* (FAB+) ---, $C_{22}H_{27}N_2O_3S_2$ requires ----; HPLC $t_r$ 3.24 min (90 : 10 = MeOH : $H_2O$).

**XDS01139 (STX750)**

Synthesised by method C. Light pink needles (260 mg; 77%). TLC $R_f$ : 0.46 $CH_2Cl_2$/EtOAc (5:1); $^1$H NMR (400MHz, DMSO-d6) 10.9 (s, 1H, $SO_2NH$), 9.25 (s, 1H, 2-H of benzothiazole), 7.95 (d, $J$ = 9 Hz ,1H, 4-H of benzothiazole), 7.92 (dd, $J$ = 8 and 1 Hz, 1H, 6'-H of benzene), 7.84 (d, $J$ = 2 Hz, 1H, 7-H of benzothiazole), 7.70 (dd, $J$ = 8 and 1 Hz, 1H, 4'-H of benzene), 7.37(t, $J$ = 8 Hz, 1H, 5'-H of benzene), 7.25(dd, $J$ = 9 and 2 Hz, 1H, 5-H of benzothiazole), 2.66 (s, 3H, 2'-$CH_3$); LC-MS 337.9 $(M)^+$; HPLC >99% $t_R$ 2.49 min (90 : 10 = MeOH : $H_2O$).

**XDS01141 (STX751)**

Synthesised by method C. Off-white solid (220 mg; 55%). TLC $R_f$: 0.83 $CH_2Cl_2$/EtOAc (5:1); $^1$H NMR (400MHz, DMSO-d6) 10.8 (s, 1H, $SO_2NH$), 7.89 (dd, $J$ = 8 and 1 Hz, 1H, 6'-H of benzene), 7.71 (d, $J$ = 8 Hz ,1H, 4-H of benzothiazole), 7.70 (d, $J$ = 2 Hz, 1H, 7-H of benzothiazole), 7.70 (dd, $J$ = 8 and 1 Hz, 1H, 4'-H of benzene), 7.36 (t, $J$ = 8 Hz, 1H,

5'-H of benzene), 7.15(dd, $J$ = 8 and 2 Hz, 1H, 5-H of benzothiazole), 3.30 (q, $J$ = 7 Hz, 2H, 2-$SCH_2$-), 2.66 (s, 3H, 1'-$CH_3$), 1.38 (t, $J$ = 7 Hz, 3H, 2-$SCH_2\underline{CH_3}$); LC-MS 397.99 $(M)^+$; HPLC 98% $t_R$ 2.90 min (90 : 10 = MeOH : $H_2O$).

**XDS01142 (STX752)**

Synthesised by method C. White crystalline solid (210 mg; 46%). TLC $R_f$ : 0.69 $CH_2Cl_2$/EtOAc (5:1); $^1H$ NMR (400MHz, DMSO-d6) 10.8 (s, 1H, $SO_2NH$), 7.88 (dd, $J$ = 8 and 1 Hz, 1H, 6'-H of benzene), 7.72 (d, $J$ = 2 Hz ,1H, 7-H of benzothiazole), 7.69 (dd, $J$ = 8 and 1 Hz, 1H, 4'-H of benzene), 7.68 (d, $J$ = 8 Hz, 1H, 4-H of benzothiazole), 7.36 (t, $J$ = 8 Hz, 1H, 5'-H of benzene), 7.15(dd, $J$ = 8 and 2 Hz, 1H, 5-H of benzothiazole), 4.25 (s, 2H, 2-$SCH_2$-), 4.13 (q, $J$ = 7 Hz, 2H, -$COOCH_2$-), 2.64 (s, 3H, 1'-$CH_3$), 1.17 (t, $J$ = 7 Hz, 3H, 2-$COOCH_2\underline{CH_3}$); LC-MS 456.0 $(M)^+$; HPLC 99% $t_R$ 2.85 min (90 : 10 = MeOH : $H_2O$).

**XDS01143 (STX753)**

Synthesised by method C. White crystalline solid (160 mg; 45%). TLC $R_f$ : 0.42 $CH_2Cl_2$/EtOAc (5:1); $^1H$ NMR (400MHz, DMSO-d6) 11.8 (s, 1H, 3-NH), 10.5 (s, 1H, $SO_2NH$), 7.81 (dd, $J$ = 8 and 1 Hz, 1H, 6'-H of benzene), 7.71 (dd, $J$ = 8 and 1 Hz, 1H, 4'-H of benzene), 7.36 (t, $J$ = 8 Hz, 1H, 5'-H of benzene), 7.28 (d, $J$ = 2 Hz ,1H, 7-H of benzothiazole), 6.93~6.98 (m, 2H, 4,5-H of benzothiazole), 2.63 (s, 3H, 1'-$CH_3$); LC-MS 353.7 $(M)^+$; HPLC 98% $t_R$ 2.32 min (90 : 10 = MeOH : $H_2O$).

**XDS01144 (STX754)**

Synthesised by method C. Off-White solid (150 mg; 77%). TLC $R_f$: 0.60 $CH_2Cl_2$/EtOAc (5:1); $^1H$ NMR (400MHz, DMSO-d6) 10.8 (s, 1H, $SO_2NH$), 7.88 (dd, $J$ = 8 and 1 Hz, 1H, 6'-H of benzene), 7.71 (d, $J$ = 8 Hz ,1H, 4-H of benzothiazole), 7.70 (dd, $J$ = 8 and 1 Hz, 1H, 4'-H of benzene), 7.69 (d, $J$ = 2 Hz, 1H, 7-H of benzothiazole), 7.36 (t, $J$ = 8 Hz, 1H, 5'-H of benzene), 7.15(dd, $J$ = 8 and 2 Hz, 1H, 5-H of benzothiazole), 3.64 (t, $J$ = 6 Hz, 2H, -$CH_2O$-), 3.50 (t, $J$ = 6 Hz, 2H, -$SCH_2$-), 3.27 (s, 3H, -$OCH_3$), 2.65 (s, 3H, 1'-$CH_3$); LC-MS 428.0 $(M)^+$; HPLC 94% $t_R$ 3.14 min (90 : 10 = MeOH : $H_2O$).

**XDS01145 (STX755)**

To a suspension of $AlCl_3$(50 mg) in DCM (5 ml) was added diethylamine( 0.4 ml). The solution was stirred under nitrogen at room temperature for 10 minutes. [6-(3-Chloro-2-methyl-benzenesulfonylamino)-benzothiazol-2-ylsulfanyl]-acetic acid ethyl ester(STX752, 100 mg) was added and the mixture was kept stirring at room temperature for 30 minutes. The reaction was quenched with water, partitioned between DCM and 5% $NaHCO_3$. The organic phase was washed with water, dried over $MgSO_4$ and evaporated in vacuo to give a yellow residue, which was purified with flash column chromatography using 20~30% ethyl acetate-DCM as elutant. 50 mg of white solid was obtained (Yield 47%). TLC $R_f$ : 0.60 $CH_2Cl_2$/EtOAc (4:1); $^1H$ NMR (270MHz, DMSO-d6) 10.7 (s, 1H, $SO_2NH$), 7.86 (d, $J$ = 8, 1H, 6'-H of benzene), 7.64~7.68 (m, 3H, 4'-H of benzene and 4,7-H of benzothiazole), 7.34 (t, $J$ = 8 Hz, 1H, 5'-H of benzene), 7.12 (dd, $J$ = 8 and 2 Hz, 1H, 5-H of benzothiazole), 4.42 (s, 2H, 2-$SCH_2$-), 3.26~3.38 (m, 4H, -$N(CH_2)_2$-, 2.50 (s, 3H, 1'-$CH_3$), 1.17 (t, $J$ = 7 Hz, 3H, -$NCH_2\underline{CH_3}$), 1.00 (t, $J$ = 7 Hz, 3H, -$NCH_2\underline{CH_3}$); LC-MS 484.0 $(M)^+$; HPLC 89% $t_R$ 2.65 min (90 : 10 = MeOH : $H_2O$).

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry or related fields are intended to be within the scope of the following claims.

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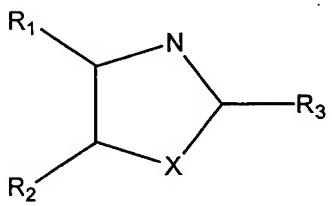
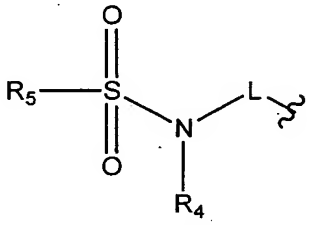
**CLAIMS**

1. A compound having Formula I



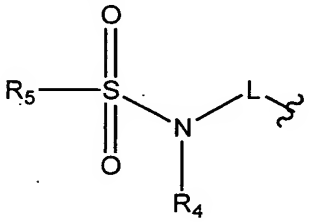
Formula I

wherein one of $R_1$ and $R_2$ is a group of the formula



wherein $R_4$ is selected from H and hydrocarbyl, $R_5$ is a hydrocarbyl group and L is an optional linker group,

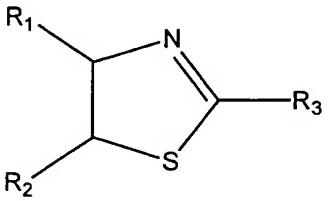
or $R_1$ and $R_2$ together form a ring substituted with the group



wherein $R_3$ is H or a substituent

and wherein X is selected from S, O, $NR_6$ and $C(R_7)(R_8)$, wherein $R_6$ is selected from H and hydrocarbyl groups, wherein each of $R_7$ and $R_8$ are independently selected from H and hydrocarbyl groups.

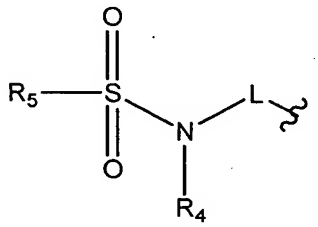
2. A compound according to claim 1 having Formula II



Formula II

3. A compound according to claim 1 or 2 wherein L is not present.

4. A compound according to claim 1, 2 or 3 wherein $R_1$ and $R_2$ together form a ring substituted with the group

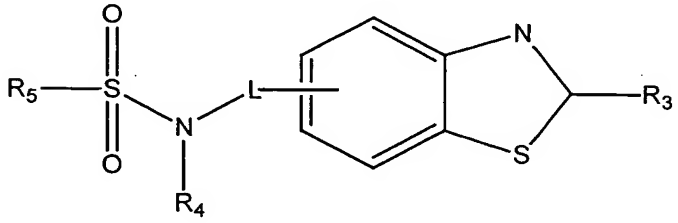


5. A compound according to any one of the preceding claims wherein $R_1$ and $R_2$ together form a carbocyclic ring.

6. A compound according to any one of the preceding claims wherein $R_1$ and $R_2$ together form a six membered ring.

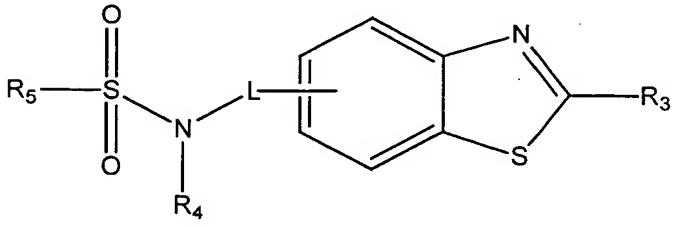
7. A compound according to any one of the preceding claims wherein $R_1$ and $R_2$ together form an aryl ring.

8. A compound according to any one of the preceding claims having Formula III.



Formula III

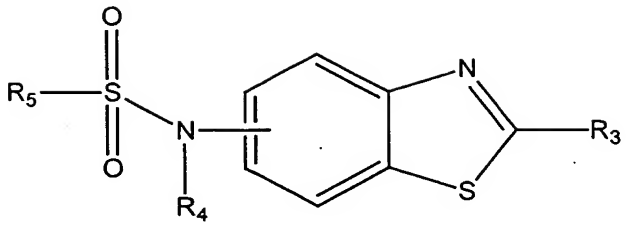
9. A compound according to any one of the preceding claims having Formula IV.



Formula IV

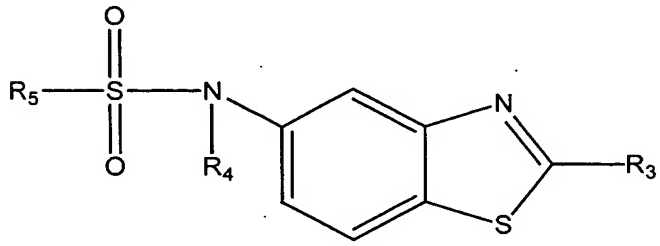
10. A compound according to any one of the preceding claims having Formula V.

Formula V



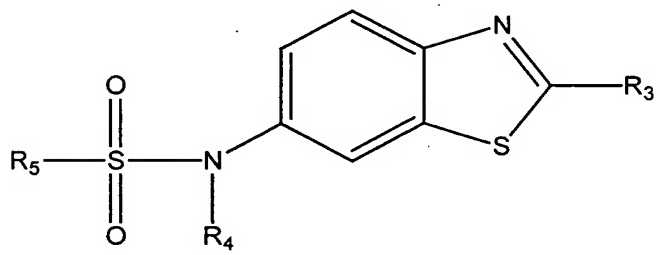
11. A compound according to any one of the preceding claims having Formula VI

Formula VI



12. A compound according to any one of claims 1 to 10 having Formula VII

Formula VII

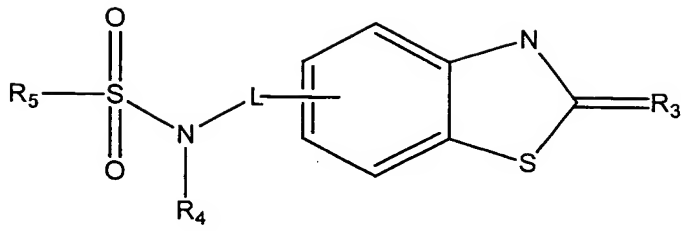


13. A compound according to any one of the preceding claims wherein $R_3$ is selected from H, hydrocarbyl, -S-hydrocarbyl, -S-H, halogen and $N(R_9)(R_{10})$, wherein each of $R_9$ and $R_{10}$ are independently selected from H and hydrocarbyl groups.

14. A compound according to any one of the preceding claims wherein $R_3$ is selected from H and $C_1$-$C_{10}$ alkyl groups, such as $C_1$-$C_6$ alkyl group, and $C_1$-$C_3$ alkyl group.

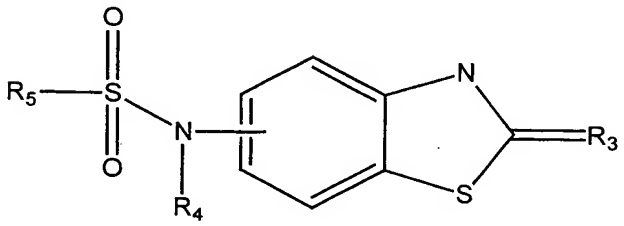
15. A compound according to any one of the preceding claims wherein $R_3$ is $-CH_3$.

16. A compound according to any one of claims 1 to 7 having Formula VIII.



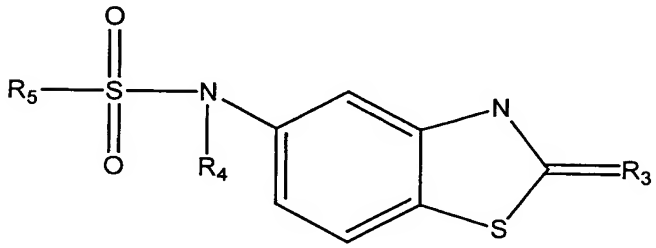
Formula VIII

17. A compound according to according to any one of claims 1 to 7 having Formula IX.



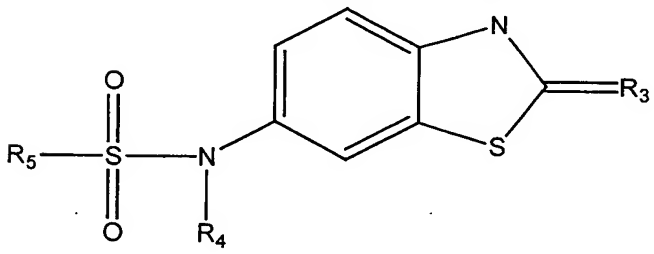
Formula IX

18. A compound according to according to any one of claims 1 to 7 having Formula X



Formula X

19. A compound according to any one of claims 1 to 7 having Formula XI



Formula XI

20. A compound according to any one of claims 16 to 19 wherein $R_3$ is selected from O, hydrocarbyl, and N($R_9$) wherein $R_9$ is selected from H and hydrocarbyl groups.

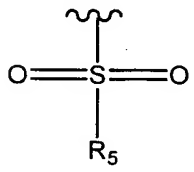
21. A compound according to any one of the preceding claims wherein $R_3$ is selected from O, $C_1$-$C_{10}$ alkenyl groups, such as $C_1$-$C_6$ alkenyl group, and $C_1$-$C_3$ alkenyl group,

NH and N-$C_1$-$C_{10}$ alkyl groups, such as N-$C_1$-$C_6$ alkyl group, and N-$C_1$-$C_3$ alkyl groups.

22. A compound according to any one of the preceding claims wherein $R_4$ is selected from H and $C_1$-$C_{10}$ alkyl groups, such as $C_1$-$C_6$ alkyl group, and $C_1$-$C_3$ alkyl group.

23. A compound according to any one of the preceding claims wherein $R_4$ is H.

24. A compound according to any one of claims 1 to 21 wherein $R_4$ is a group of the formula.



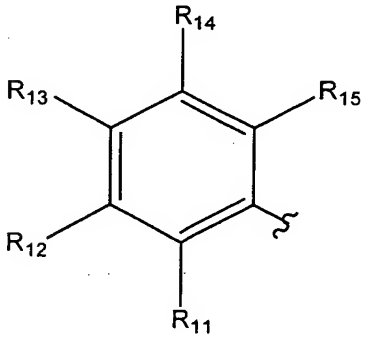

25. A compound according to any one of the preceding claims wherein $R_5$ is a substituted ring.

26. A compound according to any one of the preceding claims wherein $R_5$ is a carbocyclic ring.

27. A compound according to any one of the preceding claims wherein $R_5$ is a six membered ring.

28 A compound according to any one of the preceding claims wherein $R_5$ is an aryl ring.

29 A compound according to any one of the preceding claims wherein $R_5$ is a group having the formula



wherein each of $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ are independently selected from H, halogen, and hydrocarbyl groups.

30 A compound according to claim 29 wherein each of $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$ and $R_{15}$ are independently selected from H, halogen, alkyl, phenyl, O-alkyl, O-phenyl, nitrile, haloalkyl, carboxyalkyl, $-CO_2H$, $CO_2$alkyl, and NH-acetyl groups..

31. A pharmaceutical composition comprising a compound according to any one of claims 1 to 30 optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

32. A compound according to any one of claims 1 to 30 for use in medicine.

33. Use of a compound according to any one of claims 1 to 30 in the manufacture of a medicament for use in the therapy of a condition or disease associated with 11β-HSD.

34. Use of a compound according to any one of claims 1 to 30 in the manufacture of a medicament for use in the therapy of a condition or disease associated with adverse 11β-HSD levels.

35. Use of a compound according to any one of claims 1 to 30 in the manufacture of a pharmaceutical for inhibiting 11β-HSD activity.

36. Use of a compound according to any one of claims 1 to 30 in the manufacture of a pharmaceutical for inhibiting 11β-HSD activity.

37. A method comprising (a) performing a 11β-HSD assay with one or more candidate compounds having the formula as defined in any one claims 1 to 30; (b) determining whether one or more of said candidate compounds is/are capable of modulating 11β-HSD activity; and (c) selecting one or more of said candidate compounds that is/are capable of modulating 11β-HSD activity.

38. A method comprising (a) performing a 11β-HSD assay with one or more candidate compounds having the formula as defined in any one of claims 1 to 30; (b) determining whether one or more of said candidate compounds is/are capable of

inhibiting 11β-HSD activity; and (c) selecting one or more of said candidate compounds that is/are capable of inhibiting 11β-HSD activity.

39. A compound identified by the method according to claim 37 or claim 38.

40. A compound according to claim 39 for use in medicine.

41. A pharmaceutical composition comprising the compound according to claim 39 optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

42. Use of a compound according to claim 39 in the manufacture of a medicament for use in the therapy of a condition or disease associated with 11β-HSD.

43. Use of a compound according to claim 39 in the manufacture of a medicament for use in the therapy of a condition or disease associated with adverse 11β-HSD levels.

44. The invention of any one of claims 33 to 43 wherein 11β-HSD is 11β-HSD Type 1.

45. The invention of any one of claims 33 to 43 wherein 11β-HSD is 11β-HSD Type 2.

46. A compound as substantially hereinbefore described with reference to any one of the Examples.

47. A composition as substantially hereinbefore described with reference to any one of the Examples.

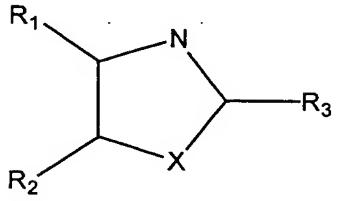
48. A method as substantially hereinbefore described with reference to any one of the Examples.

49. A use as substantially hereinbefore described with reference to any one of the Examples.

## ABSTRACT

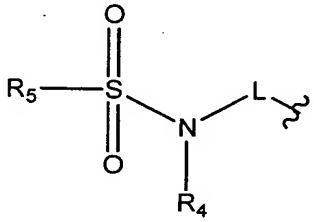
## COMPOUND

There is provided a compound having Formula I

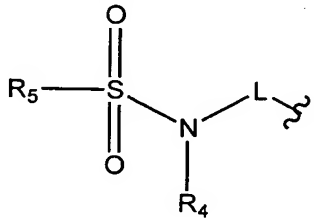


Formula I

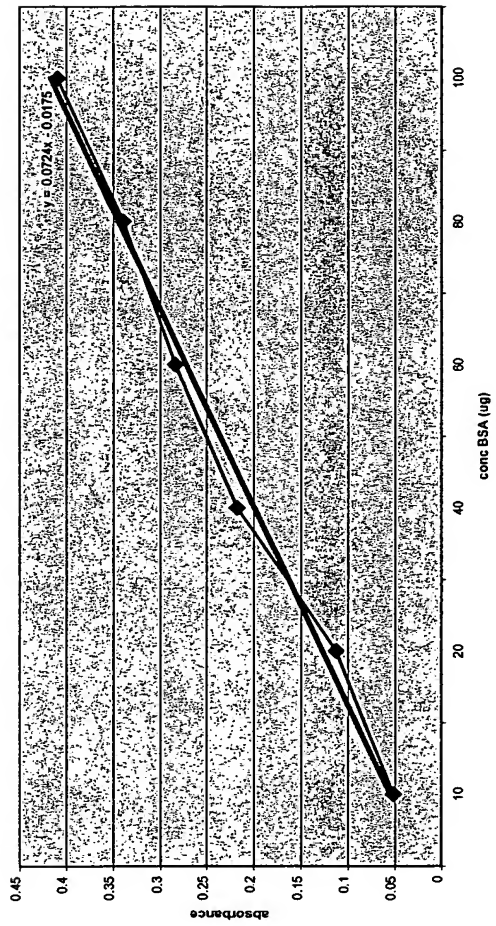
wherein one of $R_1$ and $R_2$ is a group of the formula



wherein $R_4$ is selected from H and hydrocarbyl, $R_5$ is a hydrocarbyl group and L is an optional linker group, or $R_1$ and $R_2$ together form a ring substituted with the group

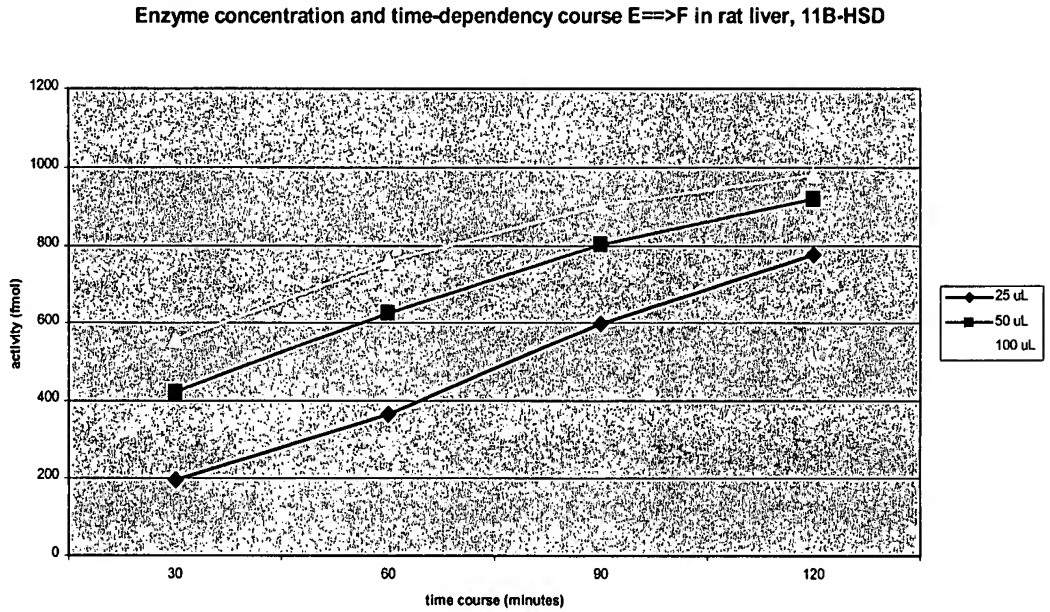


wherein $R_3$ is H or a substituent, and wherein X is selected from S, O, $NR_6$ and $C(R_7)(R_8)$, wherein $R_6$ is selected from H and hydrocarbyl groups, wherein each of $R_7$ and $R_8$ are independently selected from H and hydrocarbyl groups.



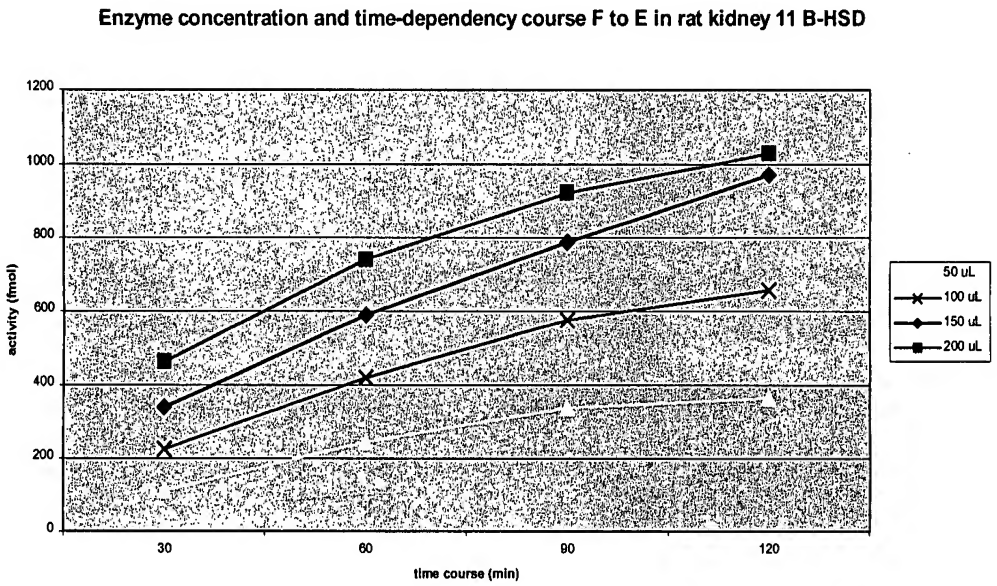

Graph 1, the amount of protein per µL of rat liver and rat kidney

Figure 1




**Graph 2, enzyme concentration and time-dependency course, E to F, in rat liver 11 β-HSD type 1 activity**

Figure 2




**Graph 3, enzyme concentration and time-dependency course, F to E, in rat kidney, 11β-HSD type 2 activity**

Figure 3